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Leprosy Review

A journal contributing to the better understanding of leprosy and its control

LEPRA

Editorial Office: LEPRA, Fairfax House, Causton Road, Colchester CO1 1PU, England
Assistant Editor: Susan Boobis, 118 Malvern Avenue, South Harrow, Middlesex HA2 9EY, England. e-mail: sboobis@aol.com

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Editor's Choice

Once again we have an issue covering a wide range of issues and there is something to interest everyone. Our review paper takes a very broad look at leprosy and I am particularly grateful to Professor Roy Curtis and Anne Ginsberg at the National Institutes of Health, Washington, USA for letting us publish the report from a meeting that was held in Washington Nov 1999. This review is stimulating, poses many interesting questions and should generate more leprosy research.

The question of relapse in multibacillary leprosy is again reviewed by Ji. He notes that even 20 years after the introduction of multidrug therapy we cannot be certain about relapse rates and suggests that monitoring of particular groups of patients will still be needed. He emphasizes the urgency of deciding how best to monitor at risk patients The paper by Lemaster *et al.* from Nepal shows what a challenge that will be, since there are no clinical features that predict highly skin smear positive cases with sufficient sensitivity and specificity. This again raises questions as to how good skin smear services can be maintained.

Pathologists are well represented in this issue with an interesting paper showing that mast cells are highest in the lesions of indeterminate leprosy (Mysorekar *et al.*) Since mast cells secrete a potent mix of cytokines and chemoactive molecules, one wonders what their role is in the events of early infection.

The paper from Professor Kumar and colleagues in Chandigarh reminds us of the importance of looking for lesions on the male genitalia, an area that is easily overlooked in misplaced modesty.

We are also starting our next major further education series, which focuses this time on HIV infection. This is a nine-part series and will cover epidemiology, the biology of HIV infection, HIV testing and counselling, management of HIV infection in a resource poor setting and clinical issues in HIV infection. I would also like to thank Professor Anthony Bryceson for being a guest editor for this series.

Diana NJ Lockwood
Editor

CALL FOR PAPERS

INTEGRATION OF LEPROSY CONTROL WITH PRIMARY HEALTH CARE

Papers are sought for a special issue of *Leprosy Review* on the topic of integration of leprosy control with primary health care.

Leprosy has traditionally been managed by specialized vertical services in most endemic countries. However, with declining prevalence and shortened treatment, it is increasingly argued that general health care staff could, and should, be able to manage leprosy without a significant increase in their workload. Integration will strengthen the ongoing decentralization processes within the health services. Successful integration of leprosy services will be an important demonstration of operational capability to achieve similar goals in other disease specific campaigns. But what will it mean for the quality of care of people affected by leprosy and for leprosy workers?

The June 2002 issue will focus on Integration, setting out the theory and practice on how effective and sustainable leprosy control can be achieved through its integration with the work of the general or primary health care system. We are eager to publish as much data as possible in this special issue and would like to encourage potential authors.

If you have data that you would like to publish in this special issue, please analyse it and write it up. Papers for this issue should be submitted by **December 2001** at the latest and will go through the usual peer review process.

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Editorial

DOES THERE EXIST A SUBGROUP OF MB PATIENTS AT GREATER RISK OF RELAPSE AFTER MDT?

It has been reported that more than 10 million leprosy patients have been cured by multidrug therapy (MDT),[1] but the definition of cure is vague. Because the clinical, bacteriological and immunological manifestations of leprosy show such wide variation, the requirements of chemotherapy are different for patients at various positions on the spectrum, and completion of MDT may or may not be equivalent to cure of the disease. Apparently, the long-term efficacy of MDT should be assessed by more solid data. For patients with multibacillary (MB) leprosy, routine clinical examination and skin smears are not sensitive enough to monitor the long-term efficacy of chemotherapy; while mouse footpad inoculation can quantify the initial 99.999% killing of *Mycobacterium leprae*,[2–4] an advanced lepromatous patient may begin treatment with a total of 10^{10} to 10^{11} viable organisms, and the failure to demonstrate multiplication of *M. leprae* at the end of treatment even in nude mice may not be taken as evidence that all viable *M. leprae* within the host have been killed. Therefore, the only way to assess the long-term efficacy of chemotherapy among MB patients is to measure the relapse rate after cessation of treatment, as is the case for other infectious diseases.

We reported in 1992 that only a single relapse was detected among 35 MB patients who had been treated with 24 months of MDT, with an overall relapse rate of 2.9%, or 0.8 per 100 patient-years, being the lowest relapse rate among the various rifampicin (RMP)-containing combined regimens that had been tested at Institut Marchoux, Bamako, Mali.[5] Because the mean duration of follow-up for this particular group of patients was only 41.9 ± 12.1 months after stopping MDT, we emphasized that it was only a provisional observation, and the patients should continue to be followed up.[5] During an additional 2.5 years of follow up, six more relapses were detected among this cohort, and the overall relapse rate had increased to 20%, or 3.3 per 100 patient-years.[6] The mean incubation period, i.e. the mean interval between stopping treatment and the appearance of relapse, of the seven relapses was 62.7 ± 18.7 months, which did not differ significantly from the mean incubation period of 58.4 ± 25.1 months among 68 relapses after stopping treatment with other RMP-containing combined regimens,[5] indicating that relapses after MDT also occurred late. All seven relapses were observed among the 18 patients who had an initial bacterial index (BI), i.e. average BI before MDT, of ≥4.0, whereas no relapse was detected among the 17 patients whose initial BI was <4.0.[6] This indicates a close correlation between relapse and the size of the bacterial population in patients before MDT, and a high initial BI of ≥4.0 appeared to be *the* most important risk factor for relapse among patients with MB leprosy. However, because the sample size was small, the unacceptably high relapse rate among this cohort in general,

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and among those patients with an initial BI of ≥4.0 in particular, must be interpreted with caution.

Recently, our observations of a high relapse rate among MB patients after MDT and its close correlation with high initial BI were confirmed by Girdhar *et al.* of the Central JALMA Institute, India, who studied a larger number of patients.[7] After a mean duration of follow-up of 3.7 years among 260 MB patients who had received treatment with 24-month MDT, 20 patients relapsed, with an overall relapse rate of 7.7%, or 2.04 per 100 patient-years. The relapse rate was 16.8% (18/107) or 4.29 per 100 patient-years among patients with an initial BI of ≥4.0, many times greater than the relapse rate of 1.3% (2/153) or 0.36 per 100 patient-years among those with an initial BI of <4.0,[7] but smaller than the corresponding figures of 38.9% or 7.0 per 100 patient-years among patients with initial BI of ≥4.0 at Institut Marchoux.[6] Most likely, the latter difference resulted from the relatively short duration of follow-up at JALMA, as pointed out by the authors.[7] Among another cohort of 301 patients at JALMA, despite treatment with MDT until skin smear negativity (mean duration 4.9 ± 2.3 years), 12 patients relapsed after a mean duration of 3.6 years of follow-up, with an overall relapse rate of 3.99%, or 1.11 per 100 patient-years; 11 of the 12 relapses occurred among patients who had an initial BI of ≥4.0. The relapse rate of 1.27 per 100 patient-years among this subgroup was significantly smaller than the relapse rate of 4.29 per 100 patient-years among the corresponding subgroup of patients treated with 24-month MDT.[7] The important difference between the results from JALMA and ours is that the relapses at JALMA occurred earlier, most of them were recorded during the first 3 years after stopping treatment (the mean durations of follow-up of the two cohorts at JALMA were only slightly longer than 3 years). A possible explanation of this difference is that relapse was diagnosed at JALMA when there was an increase in BI of ≥2+ over the previous value from any single site, with or without the appearance of new lesions,[7] whereas we diagnosed relapse only when the increase of BI was accompanied by reactivation of pre-existing lesions or the occurrence of definite new lesions, which often appeared after the increase in BI.[5,6]

The results from Institut Marchoux and from JALMA clearly suggest the existence of a high risk subgroup of MB patients who show a strong tendency of relapse after MDT. Should this subgroup be large, the strategy for leprosy elimination and the policy of chemotherapy for MB leprosy should be reviewed and, perhaps, revised. That the relapse rate among MB patients with initial BI of <4.0, who represent the great majority of MB patients in the field, was low could explain the low relapse rate in the field, even after a longer duration of follow up.[8–10]

However, the conclusions of others have been very different from that based on the data from Institut Marchoux and of JALMA. No relapse was detected among 34 previously untreated MB patients with initial BI of ≥3.0 who had been followed up at the Schieffelin Leprosy Research and Training Centre, Karigiri, India, for more than 4 years after completion of 24-month MDT.[11] Recently, the same institute reported only a single relapse among 46 patients, including the previous 34 cases, after 9.26 ± 2.98 years of follow-up.[12] Although the relapse rate was very low, it should be emphasized that the definition of high initial BI at Karigiri was ≥3.0, and that the mean value of the average BI of the patients in this group was only 3.4,[11] indicating that the bacterial load of these patients as a group was significantly lower than those designated as patients with high initial BI at Institut Marchoux[5,6] or at JALMA.[7] Gebre *et al.* from ALERT, Addis Ababa, Ethiopia, reported recently that in the AMFES cohort not a single confirmed relapse was detected among 256 MB patients who had been followed up for a mean duration of 4.3 years after completion of MDT, although 57

patients of the cohort had an initial BI of ≥4.0, and 20 of them had been followed-up for ≥5 years.[13]

Because there is no easy explanation for the deep disagreements regarding the risk of MB relapse after 24-month MDT and the existence of a high risk subgroup of MB patients who are prone to relapse, the only possible approach which may lead to reach a consensus is to collect more information from the long-term follow up of MB patients after completion of MDT. Although the 24-month MDT began to be tested in the late 1980s, it was officially recommended only in 1994[14] and widely implemented thereafter; consequently, only a few reports of the results of follow-up after 24-month MDT have been published, and the durations of follow-up were, in general, relatively short. Nevertheless, the fact that millions of MB patients have completed their treatment with 24-month MDT suggests that unpublished results of long-term follow-up, carried out either by research institutes or by routine programmes, may be available; publication of these results may help to clarify the confusion and should therefore be encouraged. To facilitate a meaningful and valid comparison of results from different sources, the following principles are proposed:

1. The follow-up should involve a cohort of MB patients who had completed 24-month MDT within the stipulated 36 months.[15] Here, MB leprosy refers to those who were clinically or histopathologically classified as lepromatous (LL), borderline lepromatous (BL), or mid-borderline (BB) by the Ridley-Jopling classification, or anyone who was BI positive at any site in the initial skin smears. For various reasons, the information published to date regarding the proportion of patients with an initial BI of ≥4.0 among total caseload or total MB patients is very limited. Data from the AMFES cohort indicate that 9% of the total previously untreated patients, or 22% of their total MB case-load, had an initial BI of ≥4.0;[13] these proportions seem unexpectedly high, and should be compared with data from elsewhere.
2. During follow-up, the patients should be examined both clinically and bacteriologically at regular intervals, preferably no less than once annually. The skin smears should be taken from the same four to six sites originally examined, and from any suspected new lesions. For those institutes or routine programs in which the patients were not followed up regularly, it may be useful to retrieve and examine a proportion of the MB patients 5 years or more after completion of 24-month MDT. The results may provide useful information regarding the risk of relapse among patients with MB leprosy.
3. Relapse was suspected if the BI at any site was found to have increased by at least 2+ over the previous value, or if any new skin lesion was detected with a BI greater than that in any pre-existing lesion. Further examinations include repeating the clinical examination and skin smears before relapse is diagnosed. Although, in its early stage, relapse may occur in the absence of obvious new lesions, and for some investigators, the occurrence of new skin lesion has not been required for the diagnosis of relapse,[7] skin lesions must occur sooner or later in real relapse, as either reactivated pre-existing lesions or entirely new lesions; also because the quality of skin smears is far below the desirable level in many control programs, we strongly recommend that relapse should be diagnosed on both clinical and bacteriological criteria: either a confirmed increase of the BI at any single site accompanied by reactivation of the pre-existing lesions, or the occurrence of definite new skin lesions with a BI higher than that in any pre-existing but non-reactivated lesions, or both. The demonstration of viable *M. leprae* by mouse footpad inoculation is helpful for confirmation of relapse, but the technique is expensive, time-consuming, and inaccessible

to the great majority of routine programs. In addition, because we had demonstrated that viable *M. leprae* were presented in nearly 90% of relapses diagnosed on the basis of clinical examination and skin smears,[5,6] mouse inoculation is not obligatory for the diagnosis of relapse.

Employing these procedures and criteria, the diagnosis of MB relapse is rather straightforward, unlike the diagnosis of PB relapse, which almost always requires differentiation from late reversal reaction. The risk of overdiagnosis of MB relapse does exist, but it is probably not very high if one follows strictly these procedures and criteria; on the other hand, the risk of underdiagnosis of MB relapse might be much greater, because of ignorance, poor quality of skin smears, insufficient duration of follow up, or a combination of all of these factors.

4. Because there was a clear tendency that more relapses were occurred with the extension of follow-up, also because the mean incubation period of relapse was at least 5 ± 2 years after treatment with 24-month MDT,[5,6] each patient should be followed for at least 5 years, and preferably for 7 years.

A number of difficulties or constraints may be encountered in attempting to follow up MB patients after completion of MDT. First, in more and more routine programs, the patients were removed from the registration as soon as they have completed MDT, and, very often, their essential records, e.g. identity, address, previous examinations and history of treatment, are missing, resulting in difficulty to retrieve the right patients for follow-up. Second, because of integration of the vertical leprosy program into general health services, the responsibility for detection of the suspected relapse cases rests upon general health workers, but a significant proportion of whom do not possess the necessary skills; in addition, the general health services often lack the manpower and resources required to follow patients who have already completed their treatment with MDT, as they are no longer considered as 'cases'.[15] Finally, because of the poor quality of skin smears in the past, and because the skin smear service very often is no longer available in the field, it is difficult to identify the MB patients who comprise the high risk subgroup and, more important, to detect the suspects of relapse. If the actions needed to overcome these difficulties are not taken as soon as possible, confusion about the magnitude of the risk of MB relapse and the possible existence of high risk subgroup of MB patients who are prone to relapse after MDT is unlikely to be resolved.

MB relapse after 12-month MDT is another important topic, because, since 1998, the great majority of MB patients have been treated with MDT for only 12 months, and there is virtually no information about the relapse rate after the shorter duration of MDT treatment. Because both the definition of MB leprosy and duration of MDT have been changed,[15] information about relapse after 12-month MDT must be collected separately from that with respect to 24-month MDT. If there exists a high-risk subgroup of MB patients who are prone to relapse after 24-month MDT, the relapse rate among patients in this subgroup may be even higher after 12-month MDT. On the other hand, relapse may nevertheless appear late, as had been demonstrated after treatment with various short course RMP-containing combined regimens.[5]

Finally, corticosteroids are being widely used for prevention and treatment of neuropathy,[16,17] and the duration of corticosteroid treatment for new neuropathy in MB patients has been recommended to continue for at least 24 weeks.[17] It remains unclear whether corticosteroid treatment may predispose to relapse of MB leprosy, or patients should be covered by chemotherapy during their treatment with corticosteroids. The experience from

the AMFES cohort suggested that corticosteroid treatment does not predispose to MB relapse, and coverage by chemotherapy is not required;[13] however, some investigators have thought otherwise.[18] Apparently, there is no consensus, and more information should be collected to clarify the possible linkage between corticosteroid treatment and relapse among MB patients, particularly among those with a high initial BI.

References

[1] World Health Organization. *Report on first meeting of the WHO Technical Advisory Group on elimination of leprosy*. WHO/CDS/CPE/CEE/2000.4.

[2] Grosset JH, Ji B, Guelpa-Lauras CC, Perani EG, N'Deli LN. Clinical trial of pefloxacin and ofloxacin in the treatment of lepromatous leprosy. *Int J Lepr*, 1990; **58**: 281–295.

[3] Ji B, Jamet P, Perani EC, Bobin P, Grosset JH. Powerful bactericidal activities of clarithromycin and minocycline against *Mycobacterium leprae* in lepromatous leprosy. *J Infect Dis*, 1993; **168**: 188–190.

[4] Ji B, Jamet P, Perani EG, Sow S *et al*. Bactericidal activity of single dose of clarithromycin plus minocycline, with or without ofloxacin, against *Mycobacterium leprae* in patients. *Antimicrob Agents Chemother*, 1996; **40**: 2137–2141.

[5] Marchoux Chemotherapy Study Group. Relapse in multibacillary leprosy patients after stopping treatment with rifampin-containing combined regimens. *Int J Lepr*, 1992; **60**: 525–535.

[6] Jamet P, Ji B, and the Marchoux Chemotherapy Study Group. Relapse after long-term follow up of multibacillary patients treated with WHO multidrug regimen. *Int J Lepr*, 1995; **63**: 195–201.

[7] Girdhar BK, Girdhar A, Kumar A. Relapses in multibacillary leprosy patients: effect of length of therapy. *Lepr Rev*, 2000; **71**: 144–153.

[8] WHO Leprosy Unit. *Risk of relapse in leprosy*. WHO Document. WHO/CTD/LEP/94.1.

[9] Li H-Y, Hu L-F, Huang W-B *et al*. Risk of relapse in leprosy after fixed-duration multidrug therapy. *Int J Lepr*, 1997; **65**: 238–245.

[10] Chen X-S, Li W-Z, Jiang C, Ye G-Y. Studies on risk of leprosy relapses in China: relapse after treatment with multidrug therapy. *Int J Lepr,* 1999; **67**: 379–387.

[11] Jesudasan K, Vijayakumaran P, Manimozhi N, Jayarajan T. Absence of relapse within 4 years among 34 multibacillary patients with high BIs treated for 2 years with MDT. *Int J Lepr*, 1996; **64**: 133–135.

[12] Shaw IN, Rao GS, Manimozhi N *et al*. A long term follow up of multibacillary leprosy patients with high BI treated with WHO multibacillary regimen for a fixed duration of 2 years. Abstracts of Presentations, *Asian Leprosy Congress*, 9-13 November 2000, Agra, India, p. 135.

[13] Gebre S, Saunderson P, Byass P. Relapses after fixed duration multiple drug therapy: the AMFES cohort. *Lepr Rev*, 2000; **71**: 325–331.

[14] World Health Organization. *Chemotherapy of leprosy*. WHO Technical Report Series no. 847, World Health Organization, Geneva, 1994.

[15] WHO Expert Committee on Leprosy. *Seventh Report*. WHO Technical Report Series no. 874. World Health Organization, Geneva, 1998.

[16] Croft RP, Richardus JH, Smith WC. Field treatment of acute nerve function impairment in leprosy using a standardized regimen—first year experience with 100 patients. *Lepr Rev*, 1997; **68**: 316–325.

[17] Saunderson P, Gebre S, Desta K *et al*. The pattern of leprosy related neuropathy in the AMFES patients in Ethiopia: definitions, incidence, risk factors and outcome. *Lepr Rev*, 2000; **71**: 285–308.

[18] Shetty VP, Ghate SD, Pai VV *et al*. Risk factors predisposing to relapse in leprosy: retrospective analysis of 78 MB and 59 PB cases presenting with relapse. Abstracts of Presentations, *Asian Leprosy Congress*, 9–13 November 2000, Agra, India, p. 136.

Bactériologie et Hygiène,
Faculté de Médecine Pitié-Salpêtrière,
91, Boulevard de l'Hôpital,
75634 Paris Cedex 13,
France

JI BAOHONG

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SPECIAL PAPER

Leprosy research in the post-genome era

ROY CURTISS III*, SALLY BLOWER**, KEVIN COOPER***, DAVID RUSSELL†,SAMUEL SILVERSTEIN†† & LOWELL YOUNG†††

** Washington University, Department of Biology, Campus Box 1137, 1 Brookings Drive, St Louis, MO 63130-4899, USA*

*** Department of Biomathematics, University of California, Los Angeles School of Medicine, 10833 Le Conte Avenue, Los Angeles, CA 90095-1766, USA*

**** Department of Dermatology, Case Western Reserve University, 11100 Euclid Avenue, LKS 3516, Cleveland, OH 44106-5028, USA*

† Department of Microbiology and Immunology, Cornell University, 5173 Vet Medical Center, Ithaca, NY 14853-2801, USA

†† Department of Physiology/Cellular Biophysics, Columbia University, 630 West 168th Street, Box 22, New York, NY 10032, USA

††† Kuzell Institute for Arthritis and Infectious Diseases, 2200 Webster Street, Suite 305, San Francisco, CA 94115-1821, USA

A workshop was held on November 21–22, 1999 in Washington, D.C. to review and discuss current understanding of leprosy as a disease and *Mycobacterium leprae* as the causative agent from the points of view of epidemiological, clinical and basic research and, based on this review, to suggest areas of needed future research. This workshop was funded by the Heiser Program for Research in Leprosy and Tuberculosis and co-sponsored by the National Institute of Allergy and Infectious Diseases. Twenty-three scientists actively engaged in leprosy research, plus two additional individuals participating by telephone conference call, contributed to the proceedings their knowledge pertaining to leprosy as an infectious disease and *M. leprae* as the causative agent. A panel of six scientists with backgrounds in epidemiology, immunology, cell biology, molecular biology and genetics, dermatology and pharmacology, but not working in basic or clinical leprosy research, evaluated the presented and published information in regard to potential future research efforts that could aid in the further curtailment of this disease.

Topics reviewed included global epidemiology, detection and diagnosis, host susceptibility, host immune responses, *M. leprae* pathogenesis, disease pathology (especially nerve damage), molecular biology, genomics, genetics and physiology of *M. leprae*, availability of

Correspondence to: R. Curtiss III

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Good Laboratory Practice (GLP)-produced *M. leprae* products as diagnostic reagents, status of current efforts to develop new skin test reagents for earlier diagnosis, and current status and prognosis of vaccine development to prevent and/or treat leprosy.

Historical background

Leprosy as a disease has existed throughout recorded history and *M. leprae* was the first etiologic agent to be implicated as the causative agent of a human infectious disease, by Hansen in 1873. Isolation of patients with the disease was the norm until the time of discovery of the first effective chemotherapeutic drug, dapsone (4,4′-diaminodiphenylsulphone), in treating the disease in the 1940s. Leprosy represents a spectrum of disease states depending upon the responsiveness or non-responsiveness of various components in the cellular immune system. The disease was classified by Ridley and Jopling, based on clinical, bacteriological, immunological and histopathological features. Tuberculoid leprosy, termed TT leprosy, is characterized by very low numbers of acid fast bacilli in skin, a few irregular non-symmetrical anaesthetic skin lesions, and an intact cellular immune response to *M. leprae*. Lepromatous leprosy, termed LL leprosy, is characterized by the presence of high numbers of bacilli in skin, many regular, symmetrical skin lesions and a significant impairment in cellular immunity to *M. leprae*, but with high titres of circulating antibodies to *M. leprae* antigens. It is individuals with the latter disease state that are thought to be most highly infectious due to the large numbers of bacilli in secretions. The majority of cases are designated borderline (BL), which represents a disease state that shares clinical, histological and immunological features with one or the other polar forms of leprosy. This state can be unstable due to immunological perturbations occurring during active disease progression. Patients initially infected with *M. leprae* often present with an indeterminate response with a characteristic skin lesion with loss of sensation, but with a minimal inflammatory response and very few bacilli. In indeterminate leprosy, in the absence of drug therapy, the disease may self-cure or progress to borderline, tuberculoid or lepromatous leprosy. In recent years, the disease states have been defined more simply as paucibacillary (PB) and multi-bacillary (MB) based on the number of lesions or the level of acid fast bacilli detected in skin biopsies to correlate with recommended drug treatment regimens.

With the emergence of dapsone resistance, first in individuals relapsing after apparent successful drug therapy and then later due to primary infection with dapsone-resistant *M. leprae*, the World Health Organization established in 1976 the special program for research and training in tropical diseases (TDR). The same year, two WHO-TDR advisory bodies were established: IMMLEP to promote the development of vaccines and THELEP to deal with the problem of dapsone resistance. In the 1960s through to the 1980s, several trials were conducted to determine whether BCG vaccination could reduce *M. leprae* infection. Interpretation of results was complicated since some studies showed no protection with others yielding significant benefit (i.e. up to 50% protection in the Malawi trial and 20–60% protection of children in one trial in India). In 1982, WHO's study group on chemotherapy for leprosy recommended the establishment of a multi-drug therapy (MDT) regimen for the treatment of individuals with leprosy. MDT involves use of rifampicin (600 mg once per month), clofazamine (300 mg once per month, and 50 mg per day), and dapsone (100 mg per day). The treatment is carried out for 12–24 months in individuals with MB leprosy and for 6 months, usually with omission of clofazamine, in individuals with PB leprosy.

The research programme to better understand *M. leprae* pathogenesis, to develop better drug therapies and to identify and test potential vaccine compositions was aided by a series of discoveries made over a number of years. A pioneering discovery by C. Shepard was the ability to infect the mouse hind footpad with *M. leprae*, which because of the low temperature of the footpad, grows 100- to 1000-fold over a period of 4–9 months. This system provided an opportunity to screen *M. leprae* for drug susceptibility. The discovery by E. Storrs and W. Kirchheimer of the susceptibility of the nine-banded armadillo (*Dasypus nomencinctus*) to infection by *M. leprae* provided a means to obtain large quantities of *M. leprae* from the spleen and liver after an 18–24 month infection period. Most recently, nude (nu/nu) mice have been used to obtain significant quantities of physiologically active *M. leprae* for detailed genetic and physiological studies. In these mice, hind footpad infection yields 10^{10} *M. leprae* in 9–12 months.

The WHO supported MDT programme was directed at the goal of reducing the prevalence of leprosy to less than 1/10,000 in endemic countries throughout the world. Toward this end, WHO recommended in 1997 an additional chemotherapeutic drug regime called ROM, which includes the administration of a single dose of antibiotics to individuals with PB leprosy with a single lesion. The single dose contains 600 mg rifampicin, 400 mg ofloxicin, and 100 mg minacycline. In 1999, the worldwide prevalence of leprosy still exceeds 1/10,000 individuals.

Global epidemiology

In considering the epidemiology of *M. leprae* infection and leprosy as a disease, it is important to define the terminology currently used by investigators in this field. Prevalence, which is equal to the number of registered cases globally or by country, is the product of the incidence of disease, which is equivalent to the number of new cases or number of detected new cases during a given interval, such as within a year, and the duration of the disease in years. It should be emphasized that the completeness of case detection and reporting varies from country to country, hence the registered case prevalence often under-represents true prevalence and new case detection underestimates true incidence. It should also be noted that individuals who have completed MDT or another drug treatment regimen and who are *M. leprae* slit-skin smear negative but nevertheless have debilitating disease, are not counted among the number of registered cases. Thus, prevalence numbers begin to equal incidence numbers when the mean duration of disease approximates 1 year. This fits quite well with the use of MDT for 6 months to cure individuals with PB leprosy and 12–24 months to cure those with MB leprosy.

Since the inception of MDT in 1982, there has been an 85% reduction in global prevalence of leprosy with the number of registered cases during the past 4 years or so, being less than 1,000,000. In 1999, the number of registered cases was 795,000 for a global prevalence of 1.4 per 10,000 individuals, a number which is in excess of the WHO year 2000 goal for leprosy control (elimination) of less than one registered case per 10,000 population. The current plateau in prevalence during the past several years is quite likely due to the fact that the number of new cases detected each year has remained relatively static or, in fact, may be increasing slightly. Thus, the number of new cases detected was 550,000 in 1985 and 795,000 in 1999 (approximately equal to the global prevalence in 1999).

Although the prevalence of leprosy is well below 1/10,000 in the majority of countries,

there are about 20 countries in which the prevalence is far in excess of this number, with India accounting for almost 80% of the registered cases globally and Brazil, Indonesia and Bangladesh having very significant numbers. It is thus of critical importance to maximize the effectiveness of identifying new cases in these high prevalence countries and enrolling these individuals in MDT programmes in a timely manner.

In evaluating the significance of these numbers and the desired goal of eliminating, if not eradicating, leprosy as an infectious disease, the panel discussed a diversity of issues. In considering infection, it is generally acknowledged that many more individuals are infected with *M. leprae* than ever develop the disease. Thus, the majority probably undergoes self-cure prior to any disease symptoms being detected. In addition, some individuals who develop symptoms usually associated with the PB form of the disease also undergo self-cure even in the absence of MDT. The time between infection and the onset of disease symptoms has very rarely been documented within several months of birth in infants born from mothers infected with *M. leprae*. Leprosy is actually rare before 3 years of age for infants born into a family with another individual with clinical disease. At the other extreme, the onset of symptoms after infection can be as long as 30 years, as evidenced by the development of symptoms in military personnel who had been exposed many years previously in a leprosy endemic area. Thus, with the time between infection and onset of disease varying from 3 to 30 years, it is evident that some of the new cases currently being identified may be due to infections that occurred years before the availability of effective MDT. Both the time for development of disease symptoms and the likelihood for development of overt disease depend on many factors, which include the route of infection, host genetic factors to be discussed below, malnutrition affecting the vitality of the immune system, and possible prior exposure to environmental mycobacteria. As with some other diseases, socioeconomic status is a significant factor. When the GNP per capita increases above $500, the incidence of leprosy decreases. An additional important risk factor is the presence of infected household contacts. Estimates range from 10% to as high as 75% of new cases occurring in such households. Thus, 25–90% of all new cases arise in individuals that must have acquired the infection by contact with an infected individual outside of the household. Although the nasal route of infection is likely, it would appear that exposure of any mucosal surface to *M. leprae* organisms and infection through skin lesions or punctures are deserving of consideration. In terms of reservoirs, most consider that leprosy is uniquely a human disease. Even though the nine-banded armadillo in the central southern United States and Mexico is frequently infected or is at least seropositive for *M. leprae*, there appears to be a very low incidence of documented transmission of *M. leprae* from armadillos kept as pets to pet owners. Nevertheless, the nine-banded armadillo, which is restricted to North America, is not likely to be a significant reservoir for *M. leprae*. More meaningful questions are how did the nine-banded armadillo become infected with *M. leprae* and do they or how might they transmit it to each other? Given their insectivorous attributes and burrowing ability, one has to question whether *M. leprae* might be a soil microorganism, at least in semi-tropical parts of the world where soil temperatures might be maintained at 30–34°C, a temperature range known to be of critical importance for the metabolic activity and survivability of *M. leprae* in the laboratory. The inability to cultivate *M. leprae* in the laboratory has precluded an evaluation of such possibilities in the past, although the methods developed by Norman Pace (*Science*, 1997; **276**: 734–740) to look at microbial ecosystem diversity by ribotyping and other methods might be amenable to identification of organisms with *M. leprae*-specific gene sequences in soil samples.

In considering these possibilities and issues, it appears that MDT may not be implemented soon enough after clinical diagnosis of disease to preclude transmission of *M. leprae* to others. The issue of whether individuals without clinical symptoms, or those with just the paucibacillary disease, exhibit infectiousness for others is unanswered. Thus, developing better diagnostic methods to identify individuals who are infected but not yet diseased, so that MDT could be initiated in a more timely manner is of paramount importance. Likewise, consideration of prophylactic drug therapy for family members in a household with disease might prove beneficial in eliminating new cases that occur in such households. In spite of the reduced prevalence, the steady or even increasing case detection caused the panel to be pessimistic about the capacity of MDT to reduce the prevalence of leprosy further in the global population, let alone to eradicate leprosy. Thus, continued research on many fronts is needed to acquire the information to improve upon MDT or seek alternative solutions to this global health problem.

Susceptibility

Although there are numerous factors that influence susceptibility to *M. leprae* infection or the likelihood that infection will lead to disease, there is evidence accruing that genetic factors may predispose individuals either to an increased likelihood of infection or influence their progression to overt disease. Thus, there is a highly significant association between the presence of the HLA-DR2 allele and leprosy in Asia and in Africa. There is also increasing evidence of leprosy susceptibility genes being present in certain families in India, although genetic loci associated with such susceptibility have yet to be identified. Recent linkage analysis data reveal a linkage between leprosy susceptibility and genetic markers on chromosome 10. This susceptibility to *M. leprae* is also shared with susceptibility to other infectious diseases. Although not adequately studied, there may be genetic differences that determine a relative propensity to the development of erythema nodosum leprososum (ENL) and other types of reactions associated with *M. leprae* infection. Results from the human genome project will contribute the framework upon which to base future studies to refine our understanding of the contribution of genetic susceptibility alleles to *M. leprae* infection and development of disease.

Immunology

The disease manifestations of leprosy represent a spectrum of immunological responses that range from Th1 to Th2 type (Th1/Th2 paradigm) similar to leishmaniasis in the mouse. For this reason, studying *M. leprae* infection and causation of disease symptoms will contribute to better understanding of human immunology. *M. leprae* infects macrophages via mannose, CR1, CR3, and CR4 receptors and scavenger receptors. Whether *M. leprae* within the phagosome secretes antigens that traffic to the macrophage cytoplasm for class I presentation and/or stimulates class II presentation from within the endosome is as yet unknown. Recent studies of macrophage processing suggest that it is likely that uptake of *M. leprae* by these cells will lead to antigen presentation by both class I and class II pathways (A. Rodriguez *et al.*, *Nature Cell Biology*, 1999; **6**: 362–368). On the other hand, the findings of Rodriguez *et al.* suggest that uptake of *M. leprae* by dendritic cells might lead these cells to present

antigen via both class I and II pathways. Both $CD4^+$ and $CD8^+$ T cells isolated from leprosy patients respond to *M. leprae* antigens and these T cells may possess $\gamma\delta$ or $\alpha\beta$ receptors. T cells have been shown to respond to 30 or so *M. leprae* antigens, but there is no information as to whether these antigens have the potential to induce protective immunity or contribute to immune dysfunction and disease progression. CD1 antigen-presenting cells are important for presentation of lipid and glycolipid antigens and play an as yet undefined role in the prevention or progression of disease. *M. leprae* infection initially promotes production of IL-12 and IL-18, the former potentiating the development of a Th1 response. In TT or PB leprosy, IL-2, IFN-γ and GM-CSF are produced. Even though the Th1 response reflected by production of these cytokines is often referred to as protective and is associated with a reasonable frequency of eradication of *M. leprae* infections, individuals expressing a Th1 type immune response may still exhibit nerve involvement and disabling disease. In LL leprosy, IL-4, -5 and -10 are produced in abundance with high antibody titres to many *M. leprae* antigens. The most striking immunological feature in LL leprosy is the *M. leprae* antigen-specific anergy in cell-mediated immunity. In tuberculoid leprosy, there is a cytotoxic lymphocyte (CTL) response in which granules containing the antimicrobial protein granulysin are likely delivered by T cells to cells infected with *M. leprae*, thereby killing these cells. In this regard, granulysin is seen in $CD4^+$ T cells in tuberculoid but not in lepromatous leprosy. This implies that the presence of granules and delivery of granulysin, whether by $CD4^+$ or $CD8^+$ T cells, is of importance in contending with *M. leprae* infection. On the other hand, there are CTL-type T cells that are double-negative for $CD4^+$ and $CD8^+$ that secrete IFN-γ and are cytotoxic. It is unclear whether these double-negative T cells have any effect on *M. leprae* infections.

Pathogenesis

Nerve damage and the consequences of nerve damage, and reactions, set leprosy apart from other diseases. The irreversible motor and sensory impairments caused by leprosy lead to increasing secondary impairments long after the disease process has been arrested. Interventions that prevent, reverse, or limit nerve impairment due to leprosy are of the greatest priority. Much headway is being made in the fundamental understanding of *M. leprae*-nerve cell interactions.

Mononuclear phagocytes ingest *M. leprae* via complement receptors (CR1 and CR3 on monocytes and CR1, CR3, and CR4 on macrophages) and fragments of complement component C3 fix to the bacterial surface. C3 binds selectively to phenolic glycolipid-1 (PGL-1), a molecule on the surface of *M. leprae*. Thus, complement receptors on macrophages, complement component C3, and PGL-1 comprise a three-component receptor-ligand-acceptor molecule system for mediating phagocytosis of *M. leprae*.

In addition to attaching to and infecting macrophages and a variety of non-phagocytic cells such as striated muscle, *M. leprae* attaches to and invades Schwann cells, the glial cells of the peripheral nervous system. *M. leprae* possesses a surface antigen that binds specifically to the G domain of the α-2 chain of laminin-2, which in turn binds to a laminin-2 receptor, α-dystroglycan, on Schwann cells. These receptors mediate the entry of *M. leprae* into Schwann cells. In LL leprosy, *M. leprae* proliferate extensively in Schwann cells of peripheral nerves, but whether *M. leprae* gene products or metabolites or the ensuing immune responses contribute to nerve damage within lesions is unknown. In any

event, *M. leprae* elicits TNF-α and IFN-γ production, both of which are associated with a strong inflammatory response.

It is important to identify specific adhesins on the surface of *M. leprae* that participate in one or more steps in the binding, entry and/or growth of *M. leprae* in macrophages and Schwann cells. Some progress has been made in this respect with the identification of the histone-like proteins and PGL-1 as key adhesins. The question of the possible expression by *M. leprae* of invasins and the means by which *M. leprae* might use signal transduction mechanisms to stimulate cells to which they attach to endocytose them are also deserving of study.

A finding of considerable clinical importance is that many individuals 2 or 3 years after initiation of MDT, and lacking detectable viable *M. leprae*, nevertheless present with significant progressive nerve damage and persistent neuritis. Whether this is due to dead bacteria or the attempt to clear dead bacteria or their components is not clear. Steroid therapy administered prior to the onset of significant nerve damage is effective in about 60% of patients to prevent these permanent disabling symptoms. Thus, other affordable immunosuppressants are currently being evaluated for improved performance in preventing disabling disease.

Immunologically mediated episodes of acute or subacute inflammation, known as 'reactions', may occur in any type of leprosy except in indeterminate leprosy and can result in deformity and disability. Most reactions belong to two main types, type II (ENL) and type I (reversal reactions). The former occurs in LL and occasionally in BL cases; the latter occurs throughout the borderline spectrum. Thalidomide has been used to treat ENL since the early 1960s, and recent studies of its mode of action have provided useful insights into the pathogenesis and immunology of reactions. Patients with active ENL have elevated serum levels of TNF-α, and thalidomide treatment rapidly reduces these levels with improvement of clinical symptoms. Thalidomide also serves to inhibit monocyte activation and inhibit T-cell activation. Thus, present-day research focuses on identifying non-teratogenic thalidomide analogues for the alleviation and understanding of ENL. Cortocosteroids (prednisolone) are the mainstay of treatment for type I reversal reactions and apparently act by switching off the Th1 response associated with reactions. In important studies, it has been demonstrated that prednisolone had little effect on the initial cellular immune response and cytokine profiles, but, by day 28, significant decreases were found in IFN-γ, IL-12, and iNOS in most patients with good clinical outcomes. These studies serve to better define the immunological basis of leprosy pathogenesis but also highlight the difficulty of modulating overactive immune responses.

Genomics and molecular biology

Sequencing of the *M. leprae* genome was initiated in 1991 and a fully sequenced and annotated genome became available in 2000. The *M. leprae* isolate sequenced came from an armadillo-passaged strain provided by the National Institute of Medical Research, Mill Hill, London, UK. The *M. leprae* genome is circular and contains 3·3 Mb compared with 4·4 Mb for *M. tuberculosis*. The *M. leprae* genome possesses 1700 open reading frames, whereas *M. tuberculosis* has 4000 open reading frames. Thus, functional gene density is considerably lower in the *M. leprae* genome than it is in the *M. tuberculosis* genome. The *M. leprae* genome contains a great deal of non-coding or pseudogene sequences and it is surprising that

this 'junk DNA' has been retained. Another obligate intracellular parasite, *Rickettsia,* also has pseudogenes in its genome, although the 10% pseudogene content reported is less than for *M. leprae* where close to one-half of the genome is composed of non-coding pseudogene sequences. Also, there are fewer insertion sequences, but more repetitive DNA sequences (RLEP, REPLEP, and LEPREP) in the *M. leprae* genome than in the *M. tuberculosis* genome. A comparison of coding sequences shows there are proteins that have 35–95% amino acid identity between *M. leprae* and *M. tuberculosis* or BCG. The latter observation probably explains why BCG vaccination is sometimes partially effective in preventing *M. leprae* infection.

There appear to be very few differences in *M. leprae* genomes from different strains and this genome sequence conservation has hampered studies on transmission of specific strains within communities or countries. Only a single polymorphism has been found among *M. leprae* strains and this is due to the presence of two copies of the repetitive sequence RLEP linked to the *polA* gene leading to inversion of *polA* in some strains as opposed to others. (There are at least 30 copies of the RLEP sequence scattered throughout the *M. leprae* genome.) Hopefully, the availability of the complete sequence of the *M. leprae* genome will facilitate the discovery of additional polymorphisms that will facilitate epidemiological studies and enable tracing the chain of transmission of specific *M. leprae* strains in humans.

The constancy of the *M. leprae* genome and especially the maintenance of such an array of pseudogenes in the absence of any apparent benefit is most surprising. Quite possibly the exceedingly long generation time of 2 weeks or so and the ability to prosper in phagocytic cells designed to kill microorganisms by generation of free radicals and production of a diversity of antimicrobial compounds has selected for the inordinate ability of *M. leprae* to repair any and all genetic damage that may arise spontaneously or due to any of these insults. Such conjectures can be tested by using microarray technology and other means to identify genes expressed by *M. leprae* within specific cell types and by defining those gene products that enable *M. leprae* to attach to, invade and survive in macrophages and Schwann cells.

Physiology and genetics

M. leprae frozen at −80°C and then thawed has greatly reduced viability when assayed for growth in mouse footpads and greatly reduced metabolic activity as measured by CO_2 generated from oxidation of radioactive palmitate, a quantitative assay for measuring subtle changes in metabolism. Whether physiologic impairments consequent to freezing are repaired once *M. leprae* enters an animal host remains to be determined. Even so, for studies of organisms maintained at 32–33°C, it seems preferable to use microorganisms freshly harvested from nude mice. In humans and mice, *M. leprae* prefers the cooler parts of the body, while being able to multiply systemically in the armadillo, whose body temperature is 33–34°C. In accord with these observations, metabolic activity is markedly impaired by incubation of *M. leprae* at 37°C. Storage at 4°C has the least adverse effect on *M. leprae* physiologic activity measured at 32–33°C. Physiologically active *M. leprae* incubated under optimal physiologic conditions are able to take up and incorporate glucose, 6-phosphogluconate, glycerol (into lipids), amino acids into proteins and purines (hypoxanthine and adenine) and pyrimidines (cytosines and thymidine) into nucleic acids. Pyrimidines are incorporated into nucleic acids less efficiently than purines. Inorganic phosphate is also incorporated into macromolecules. Palmitate, in addition to its oxidation, is very efficiently

incorporated into PGL-1 and PDIM (phthiocerol dimycocerosate) but acetate and pyruvate are incorporated into macro molecular constituents very inefficiently. Unlike *Rickettsia*, *M. leprae* is unable to take up phosphorylated nucleotides. On the other hand, *M. leprae* take up and incorporate nucleosides more efficiently than purine and pyrimidine bases. Enzyme assays on metabolically active *M. leprae* indicate a functional tricarboxylic acid (TCA) cycle and glycolysis via the Embden-Meyerhof and hexose monophosphate shunt pathways. Work to confirm and extend studies with armadillo-derived *M. leprae* is in progress using nude mouse-derived bacteria.

In terms of the very slow growth rate of *M. leprae*, there is some suggestion that it is defective in NADH metabolism. This defect, in combination with its content of only one copy of genes encoding ribosomal RNA, may contribute to a very long generation time. It is now possible to examine questions such as these by using *M. leprae* maintained under metabolically active conditions.

M. leprae cells can be infected with bacteriophage AE129 engineered to express luciferase. The finding that luciferase activity in these phage-infected cells can be inhibited by anti-leprosy drugs offers an opportunity to undertake new types of research. Efforts are in progress to introduce the green fluorescence protein gene (*gfp*), which will facilitate microscopic tracking of *M. leprae* in animal tissues or in cells in culture. Analysis of the *M. leprae* genome may suggest reasons for the inability of *M. leprae* to grow axenically and for its slow growth rate. The ability to introduce foreign DNA into *M. leprae* will allow investigators to test directly a variety of hypotheses by complementing *M. leprae* genes or by supplementing *M. leprae* with genes from other mycobacteria. Such introduced genetic information could be on a phage genome to be incorporated into the chromosome or more likely to replicate on a phasmid, cosmid or plasmid replicon within *M. leprae* cells. Thus, coupling new evolving gene transfer procedures with the array of data discernible from genomic analysis should accelerate the rate of progress in understanding *M. leprae* growth, infection, pathogenesis and distribution.

The availability of the sequence of all complete ORFs in the *M. leprae* genome, combined with the power of modern proteomics (two dimensional polyacrylamide gel electrophoresis, selective ion monitoring and other forms of mass spectrometry) has resulted in the realistic expectation that the entire proteome will soon be defined. Already, specific cationic proteins involved in Schwann cell interaction and a modest complement (as compared to *M. tuberculosis*) of lipoproteins involved in Th1 and Th2 immune responses have been defined.

Anti-leprosy drugs and drug resistance

With the commenced use of sulpha drugs for chemotherapy of bacterial diseases in the 1930s, a di-substituted sulphone (Promin) was tried for treating leprosy in Carville, LA in 1941, with encouraging results. Promin was relatively expensive and required intravenous administration, so other sulphone drugs were tried during the 1940s, including orally administered dapsone. Dapsone therapy was introduced in India, Nigeria and Brazil in the late 1940s and was shown to be very effective in treating leprosy in all three countries. Since dapsone was at that time only useful for treating patients with leprosy, and since most of these patients were too poor to pay for drugs, there was effectively no market for it. Consequently, dapsone was available for governmental use at a very low cost. In this regard, dapsone is inexpensive (as cheap as aspirin), stable, and relatively non-toxic. Nonetheless, *M. leprae* infections with

secondary and then primary dapsone resistance appeared leading WHO to adopt the MDT program in 1982. This programme was based on the premise that the use of three drugs (rifampicin, clofazamine and dapsone) would preclude the development of resistance to any one of the component drugs used in MDT. It should be noted that rifampicin was chosen as one of the three drugs because of its bacteriocidal effect on *M. leprae*, but also because it was no longer under patent protection thereby decreasing its cost. Clofazamine was also inexpensive. Thus, all of the three drugs used in MDT as well as the drugs for the ROM regime, are donated by drug manufacturers (especially Novartis) free of charge. The World Health Organization, Nipon Foundation and the International Federation of Anti-Leprosy Associations distribute these drugs. There are new macrolide and fluoroquinolone drugs that are highly active against *M. leprae* but because of cost are seldom used to treat leprosy in Africa, Asia and South America. These new drugs are likely to enter the treatment regimen only if they become inexpensive or are proven effective after a very brief treatment regimen or if drug resistance causes one or more of the existing drugs in the MDT repertoire to become ineffective. In this regard, it is known that rifampicin resistance can arise quite readily if used alone and ofloxocin-resistant *M. tuberculosis* has arisen in India. Dapsone resistance has long been known, but its mechanism has only recently been elucidated. In this last regard, it is not known whether use of MDT during the past 18 years has altered the frequency of dapsone-resistant *M. leprae*.

The availability of methods for research with metabolically active *M. leprae* along with the ability to use luciferase or other reporters should facilitate screening of new drugs for effectiveness in killing *M. leprae*. In this regard, the HIV epidemic has stimulated efforts to discover and evaluate new drugs for the control of *M. tuberculosis* and of the *M. avium* complex. Some of these new drugs are retained in tissues quite well and might be effective for short-term treatment regimens and in alleviating some of the problems associated with *M. leprae*'s neurotropic propensity and adverse sequelae that arise as a consequence. Although the inclusion of three drugs in the MDT programme is wise, it must be surmised that at some time multiple drug resistance will arise, especially if the incidence of new cases does not decline. Multidrug resistance would pose a significant impediment to the eradication of leprosy.

With the sequence of the *M. leprae* genome, it now becomes possible to use specific oligonucleotides and PCR to recognize mutations for resistance to rifampicin, ofloxicin, dapsone, etc. The problem with such PCR methods is that they must be done in an institution with the technical capabilities for such assays. Nevertheless, such tests can be done with small samples of materials sent to a central testing institution and this would likely be as efficient an approach to monitor drug resistance as taking biopsy material, inoculating footpads of nude mice, recovering sufficient cells and using a phage infection luciferase activity to screen for drug resistance or sensitivity.

Diagnosis and diagnostic reagents

The diagnosis of leprosy is still largely based on clinical symptomology and occasionally skin biopsies to verify the presence of acid-fast bacilli. It has been desired for many years, however, that some diagnostic method be developed that would detect infection and the presence of multiplying *M. leprae* as soon as possible before clinical signs are apparent. When PGL-1 was identified as a *M. leprae*-specific antigen, efforts were directed at using the

presence of antibodies to this molecule as a test for occult infection. Such antibodies can be detected prior to the onset of clinical symptoms. Anti-PGL antibodies, which are largely IgM, are quite prevalent in LL or MB patients but are not prevalent in TT or PB patients. Thus the detection of PGL-1 or antibodies to it became less attractive as an early diagnostic indicator.

Another immune reactivity test involves intradermal inoculation of an autoclaved extract of armadillo-derived *M. leprae*, the so-called lepromin test, and is read at 3 weeks. The lepromin test is not analogous to the tuberculin test, which is read as a DTH reaction to soluble *M. tuberculosis* PDP 48 h after injection, and has no value as a diagnostic test for subclinical leprosy. However, the lepromin test can be a prognosticator for the type of leprosy a person is likely to develop (i.e. borderline tuberculoid or tuberculoid if the test is positive and borderline lepromatous or lepromatous if the test is negative).

Two new approaches are being taken to develop improved skin test antigens. In one such approach, armadillo-derived *M. leprae* are fractionated to generate a set of protein antigens associated with the cell wall in one case and from the cytoplasm in another. These protein preparations are purified to remove all non-specific immunosuppressive lipoglycans and lipids and these procedures are done under GLP conditions to satisfy US Food and Drug Administration requirements for an IND (Investigative New Drug) to conduct a phase I/II trial in a leprosy endemic area. As this research progresses, there will be an integration between *M. leprae* genomics and proteomics wherein 2D polyacrylamide gel electrophoresis is being used to identify *M. leprae* proteins encoded by the 80 or so genes unique to *M. leprae* and not present in *M. tuberculosis*. (Of course, these *M. leprae*-specific genes will have to be first evaluated for their absence in a diversity of other mycobacterial species, including those from soil.)

In another approach, peptide antigens representing *M. leprae*-specific epitopes have been suggested by comparison of *M. leprae* genome sequences with those from other mycobacteria. Some of these peptides seem likely to be *M. leprae*-specific by detection of T cell responses to them in the blood of patients with leprosy, but not by T cells from individuals previously vaccinated with BCG or with tuberculosis. It is likely, however, that some of the epitopes on these peptides may be MHC-restricted and thus give rise to T cell responses in only some patients. Nevertheless, the evaluation of many such peptides should enable the preparation of a cocktail that would be recognizable by one or more T cell clones present in the blood of most patients with leprosy, provided they are capable of making a T cell response to leprosy antigens. Whether the diagnostic methods under test will reveal infection long before clinical symptoms are recognizable is unknown. If so, then MDT can be initiated sooner with an increasing likelihood that both the prevalence and incidence of leprosy can be further reduced.

Vaccines and vaccination

BCG vaccination in Malawi and India, at least in some studies, has resulted in a reduction in the incidence of leprosy. Now that the genetic relationship between the various BCG vaccine strains is known, the leprosy research community can evaluate the success or non-success of various BCG vaccination trials, whether in controlling tuberculosis or leprosy, with the particular properties of the BCG strain used as the vaccine in that particular trial. This information would be useful for both *M. tuberculosis* and *M. leprae* control efforts, since those desiring to develop a vaccine against *M. tuberculosis* are endeavouring to specifically

attenuate *M. tuberculosis* and *M. bovis* and would hopefully choose parent strains that would have the potential for inducing cross-protective immunity against both *M. tuberculosis* and *M. leprae*.

A prophylactic leprosy vaccine trail conducted in South India in the early 1990s, comparing four vaccines (BCG, BCG and killed *M. leprae*, and two cultivatable Indian mycobacterial strains, W and ICRC), yielded promising results. Although it was possible to assess the overall protective efficacy of the candidate vaccines against leprosy in the study population, the observed incidence rates of leprosy were not sufficiently high to ascertain the protective efficacy of the candidate vaccines against progressive and serious forms of leprosy. Protection observed with the ICRC vaccine and the combination vaccine (BCG and killed *M. leprae*) meets the requirement of public health utility. However, the killed *M. leprae* vaccine is unlikely to be available in the future, but the ICRC vaccine is readily available and might be considered for more widespread implementation.

With the prevalence of leprosy approaching 1/10,000, it would seem unlikely that a prophylactic vaccine would be highly cost-effective in preventing leprosy. Thus, the community pressure to vaccinate would not be sufficiently large, except maybe in localized highly endemic areas within countries with a high prevalence and incidence of leprosy. On the other hand, a vaccine that would prevent *M. tuberculosis* infection as well as *M. leprae* infection would likely enjoy a much higher use and thus generate a more satisfactory outcome. By comparative analysis of the *M. leprae* and *M. tuberculosis* genomes, it may be possible to identify antigens that are highly cross-reactive and which might generate a protective immune response, specifically a CTL, Th1-dependent type of immunity. The induction of mucosal and systemic immune responses directed at relevant surface antigens of *M. leprae* and of *M. tuberculosis* might contribute significantly to decreasing the likelihood of infection, especially since *M. tuberculosis* is a respiratory pathogen and there is considerable favor for this idea as a means of transmission of *M. leprae*.

Basic and clinical leprosy research community

The perceived success in eradicating leprosy coupled to the increasing global concern for tuberculosis, which has been very much augmented by the HIV epidemic and the development of drug-resistant *M. tuberculosis,* have caused many mycobacterial researchers previously working on leprosy to switch to working on tuberculosis. Thus, the National Institutes of Health is currently funding two contracts to provide much-needed *M. leprae* research resources and five investigator-originated research projects on various aspects of leprosy and *M. leprae* biology. The WHO TDR spends less than 2% of its total budget on leprosy and most of this is concerned with managing the MDT programme. There has been a similar decline in the number of laboratories globally conducting basic research on leprosy and this situation is not conducive to generating the types of knowledge needed to contend with this dread disease and to effect its eradication. It should be emphasized that the members of the small leprosy research community collaborate extensively with one another sharing clinical materials, research reagents, and importantly, exchanging personnel, some of who receive training in research laboratories and then return to clinical institutes. The Armauer Hansen Research Institute in Addis Ababa, Ethiopia; the Central JAMA Institute for Leprosy in Agra, India; the Aga Khan University in Karachi, Pakistan; the Leonard Wood Memorial Research Center in Cebu City, Philippines; the Oswaldo Cruz Foundation with laboratories in

Rio de Janeiro, Brazil and the Anandaban Hospital in Kathmandu, Nepal, all located in countries with endemic disease, facilitate these ongoing research efforts. In addition, there are central leprosy research laboratories in many countries throughout the world that can be tapped for intellectual as well as clinical and research resources. Increased funding for leprosy research is needed and will be efficiently used by the cadre of well-trained and highly committed investigators currently working in this field.

Conclusions and recommendations

Based on the foregoing, the panel reached the following conclusions and recommendations.

1. It makes economic and human sense to eradicate leprosy as a global problem. This is an unobtainable goal with the current knowledge and tools and dependence entirely upon the use of multiple drug therapy as presently carried out. An intensification of a much better funded research enterprise on *M. leprae* and leprosy is therefore necessary.
2. Investigation of the epidemiology, natural history and transmission of leprosy should be given the highest priority. These studies will require development of improved DNA reagents, diagnostic materials and diagnostic tests suitable for use in endemic countries. The roles of animal, and even soil, reservoirs for *M. leprae* need to be determined since such information is essential to develop a plan for control and possible eradication of leprosy.
3. The materials and information required for modern molecular epidemiology and for *M. leprae* detection in potential reservoirs will likely soon be available from analysis of the *M. leprae* DNA genomic sequence. Further improvements of means for genetic manipulation of *M. leprae* coupled with studies of *M. leprae* under physiological conditions will allow identification of gene products required for pathogenicity. Identification of gene products expressed during infection will establish mechanisms for disease-associated pathogenesis and allow the development of better diagnostic reagents and vaccines. For these reasons, research on *M. leprae* genomics, molecular biology, genetics and physiology should have a high priority.
4. The continued availability of standardized, high-quality research materials including, but not limited to, armadillo- and mouse footpad-derived *M. leprae*, purified and characterized cell constituents and proteins, both native and recombinant, antibodies and genetic constructs will be invaluable for future research progress.
5. Research on tuberculosis and *M. tuberculosis* is often relevant to the understanding of leprosy and *M. leprae*. For this reason, research on leprosy should take guidance from tuberculosis research in attempting to confirm important hypotheses without being merely duplicative. This will be a more cost-effective approach and focus leprosy research on issues that are unique to the disease and to the causative pathogen.
6. Since multiple drug therapy can eliminate the infection in the individual, but has little or no effect on subsequent pathology associated with reactional states and progressive nerve damage leading to deformity, it is imperative that further research be conducted on the basic mechanisms of immunological reactions and nerve damage in leprosy to develop interventions that prevent such damage in *M. leprae* infected individuals. Collaborations between leprosy researchers and neuroscientists should therefore be fostered.
7. The current means to study physiologically active *M. leprae* should enable evaluative screens of new drugs for effectiveness in leprosy control, especially those now being

developed and tested for efficacy against *M. tuberculosis*. These drugs and/or drug combinations can then be evaluated in the mouse footpad model.

8. The ultimate goal of controlling and even eradicating leprosy is likely to be dependent on development of a safe efficacious vaccine that prevents infection, especially infection by both *M. tuberculosis* and *M. leprae*. Acquiring basic knowledge of the immune responses needed to confer protection and identifying the *M. leprae* antigens that elicit these responses is a high priority. In addition to a preventative vaccine, consideration should be given to the development and evaluation of a vaccine with therapeutic potential in treating patients with leprosy.

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Prediction of 'highly skin smear positive' cases among MB leprosy patients using clinical parameters

JOSEPH W. LEMASTER*, TIN SHWE**, C. RUTH BUTLIN*** & PAUL W. ROCHE***

**Department of Family Medicine, University of Washington, P.O. 354696, Seattle, Washington (WA) 98195*

***Leprosy Control Division, HMG, Nepal*

****Mycobacterial Research Laboratory, Anandaban Leprosy Hospital, PO 151, Kathmandu, Nepal*

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Summary Although 'highly skin smear positive' MB leprosy cases are known to be at high risk of relapse after release from treatment, and have been recommended to receive 'prolonged duration' MDT, government field-based control programmes without skin smear facilities have no simple alternative method to detect such cases. This study reports a significant prevalence of 'highly smear positive' cases amongst 2374 new multibacillary cases recently surveyed by skin smears in Nepal, and retrospectively analyses 555 newly detected, previously untreated BL and LL cases to identify clinical and laboratory parameters that may be associated with a 'highly positive skin smear'. While some parameters showed high sensitivity in predicting 'highly positive smear' status, none showed both high sensitivity and high specificity simultaneously.

Introduction

Recently the WHO Expert Committee on Leprosy has concluded that the length of fixed duration multi-drug therapy (MDT) for multibacillary (MB) patients could be shortened to 12 months, without greatly increasing the risk of relapse for the majority of such patients.[1] There has been much debate over this topic in the last year among national control programmes, who have the responsibility for implementing this decision in their own countries. The risk of relapse among MB leprosy cases who have received the standard 24 months of MDT has been found to be as low as 0·77%[2] and as yet, there is no evidence that shorter courses of therapy will increase the risk of relapse.[3,4] However, a high rate of relapse (39%) has been reported among MB patients with 'highly positive slit skin smears' (bacterial index (BI) $\geq$ 4.0+) treated with 24 months of standard MDT.[5] Pattyn in Zaire and others have suggested that the

Correspondence: J. W. LeMaster (e-mail: lemaster@gateman.com)

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higher the BI, or the shorter the duration of therapy, the higher the risk of relapse,[6,7] and many programmes wish to treat highly smear positive (HSP) patients with at least 24 months MDT.

In Nepal, a diagnosis of MB leprosy is based on clinical findings, not on slit skin smear results. Newly detected cases in Nepal who have disease in more than two body areas are classified as multibacillary, and until recently all were given 24 months fixed duration MDT. Despite the national policy to treat HSP cases with 24 months of MDT, there is no skin smear referral system available throughout in Nepal. Government peripheral health posts must continue to classify cases on the basis of clinical findings, and from 1999 have treated all MB cases with the 12-month MDT regimen.

In 1999, slit skin smears were taken from 2374 new previously untreated MB cases representing about 10% of the new cases detected during a national leprosy elimination campaign. At the time of skin smear, all cases had 1–6 months of MDT treatment. Of these patients, 18·2% (433) were found to be skin smear positive, and 8·2% (193) had either an average BI > 3·0, or at least one lesion with BI ≥ 4·0 (Nepal Leprosy NGO Network meeting minutes, August 1999). Thus, there is a substantial proportion of HSP MB cases in field programmes in Nepal, but without skin smear services there is no way to identify these high-risk cases in the field-based programme.

This study attempts to identify whether a particular clinical feature or combination of features can be used in a field setting to identify cases with 'highly positive' skin smears.

Materials and methods

We used data collected retrospectively from a previously described sample of MB cases.[8] Data from 649 previously untreated MB patients diagnosed on clinical and bacteriological grounds, who presented to Anandaban Leprosy Hospital between 1989 and 1997 were used. Ninety-four cases were excluded from the analysis because an initial skin smear had not been performed. The remaining 555 cases provided the data for this analysis. Clinical, bacteriological and serological information collected at the time of first presentation and diagnosis was reviewed. Slit skin smears were taken from all 555 cases, and the mean BI was calculated from smears taken from a minimum of four sites including a clinical lesion. The Disability Index (DI) according to the WHO classification system was recorded for 460 patients. In addition, the number of body areas with disease was recorded for 550 cases, based on the number of areas in which there were either skin patches or enlarged nerves. The maximum number of body areas possibly affected was nine, consisting of four for the trunk (divided anterior and posterior sagittally), four for the limbs and one for the head. The total number of skin patches (for 364 patients), enlarged, palpable nerve trunks (for 529 patients), the presence of diffuse infiltration and of lepromatous (non-ENL) nodules (both for 555 patients) was also recorded. IgM anti-PGL-1 antibodies were measured from serum in 450 cases. We also recorded the patient's estimate of the numbers of years since the first appearance of leprosy disease: this gave a measure of the period of untreated disease before treatment started. ENL reactions at presentation were also recorded.

Differences between HSP and other cases with respect to quantitative variables were tested using the Mann-Whitney *U*-test. Unadjusted odds ratios and 95% confidence intervals were calculated from two-way contingency tables, comparing the proportions of those with and without HSP for each clinical characteristic examined. Multiple logistic regression was used to control for confounding between the predictors, and to calculate adjusted odds ratios

Table 1. Relationship between clinical and laboratory parameters and the prevalence of highly positive skin smears (BI ⩾ 4+)

Predictor	No. HSP/total positive (PPV)	No. HSP/total negative (NPV)	Unadjsuted OR (95% CI)	Adjsuted OR (95% CI)	Sensitivity (%)	Specificity (%)
LL classification	137/211 (65%)	78/344 (23%)	6.3 (4.1–9.6)**	6.8 (3.0–15.8)***	64	78
Age > 40 years	89/246 (36%)	126/308 (41%)	0.81 (0.6–1.1)	n/a	41	54
Male sex	167/417 (40%)	48/137 (35%)	1.2 (0.8–1.8)	n/a	78	26
Body areas with disease > 5	204/485 (42%)	11/65 (17%)	3.6 (1.8–6.9)***	10.1 (2.3–43.8)**	95	16
Enlarged nerves > 4	167/364 (46%)	42/165 (25%)	2.4 (1.6–3.7)***	1.0 (0.4–2.5)	80	39
Skin patches > 5	48/195 (25%)	88/169 (52%)	0.3 (0.19–0.47)***	0.7 (0.3–1.5)	35	35
Skin infiltrate	193/364 (53%)	22/191 (12%)	8.2 (4.9–13.9)***	3.7 (1.5–9.0)**	90	50
Skin nodules	68/99 (69%)	147/456 (32%)	4.6 (2.8–7.5)***	1.9 (0.8–4.9)	32	91
anti-PGL-1Ab sero-positive	152/319 (48%)	29/131 (22%)	3.2 (2.0–5.1)***	1.7 (0.7–4.1)	84	38
ENL reaction (at presentation)	18/33 (24%)	197/522 (38%)	2.0 (1.0–4.0)*	1.3 (0.3–5.1)	31	88
Established disability	33/148 (22%)	142/312 (46%)	0.35 (0.22–0.54)***	0.4 (0.2–0.9)	19	60

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$. PPV = positive predictive value NPV = negative predictive value.

for those cases for which all clinical and laboratory parameter data was available (291 cases). Specificity, sensitivity, and positive predictive value for a diagnosis of HSP were calculated from each predictor or two-way combination of predictors.

Results

All information was collected at the time of diagnosis. Of the cases in the sample, 344 (62%) were classified as borderline lepromatous (BL) on clinical grounds at first presentation and 211 (38%) as polar lepromatous (LL). Prevalence odds ratios describing the risk relationships between clinical and laboratory parameters and HSP status are shown in Table 1. Patients with highly positive skin smears (≥4+) had significantly more body areas with leprosy disease, more enlarged nerve trunks and higher levels of anti-PGL-1 antibodies than patients with skin smears with lower BI.

We analysed each clinical feature to assess the strength of association with classification of a case as HSP, and calculated the sensitivity, specificity, and positive and negative predictive values for a diagnosis of an HSP skin smear. An LL classification, the presence of skin infiltrate, the presence of lepromatous nodules, the presence of ENL, anti-PGL-1 antibody seropositivity, more than five body areas affected by leprosy, and more than four nerves enlarged, all predicted significantly increased risk of a case being HSP. The presence of six or more skin patches or a DI > 1 both predicted a decreased risk for HSP. Neither age greater than 40 years nor male sex was predictive of HSP.

Because a number of the clinical features shown in Table 1 appeared to be strongly predictive of HSP, we used a logistic regression model to control for potential confounding amongst the most strongly predictive features. These 'adjusted odds ratios' are also shown in Table 1. In this model, having more than five body areas with disease was the most sensitive predictor, whereas the presence of lepromatous nodules was the most specific. No single predictor simultaneously showed a sensitivity and specificity in excess of 64%.

The three clinical features, LL classification, more than five body areas with disease and skin infiltration, were combined to measure the effect on HSP prediction. In each case, if *either* one or the other of the two clinical features was present, HSP was predicted. Despite odds ratios that were generally stronger than for either clinical feature on its own, the sensitivity of these combinations was high, up to 98%, but specificity and positive predictive values were low.

Table 2. Effect of combinations of clinical characteristics in predicting high skin smear positivity

Symptoms	Odds ratio (95% CI)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)
More than five body areas *or* skin infiltration	7.7*** (2.2–25.9)	98	9.8	41
More than five body areas *or* LL class	1.4 (0.6–3.2)	96	6.1	39
Skin infiltration *or* LL class	1.7 (0.7–3.9)	96	5.9	39
Any feature positive	3.6 (0.8–16.4)	99	3.2	39
All features positive	0.9 (0.6–1.3)	29	68	37

*** $P < 0{\cdot}001$.

Combination of all three clinical features in Table 2 did not yield any additional advantage. As one might expect, if *any* of the three features was accepted as predictive of HSP status, sensitivity approached 100%, while specificity fell towards 0%. The opposite was true if we required the simultaneous presence of *all* three features to predict HSP status: increases in specificity were at the cost of decreases in sensitivity.

Discussion

The practical utility of the clinical features examined here in identifying HSP cases is marginal. No single clinical feature simultaneously showed both sensitivity and specificity in excess of 65%. This was also true of all the combinations of clinical features examined. However, some of the single features and some of the combinations of features did show very high sensitivity. Control programmes must examine their priorities in order to interpret and use these results. If the highest priority is given to identifying virtually all HSP cases, and the unnecessary treatment of up to 50% of those who are not, in fact, 'highly smear positive' is acceptable from a logistical and cost basis, a programme might choose to use one or more of the most sensitive clinical features identified here as a screening tool. However, the usual reality for control programmes is that they must conserve scarce resources, making sure that a majority of those predicted to be HSP are so before providing additional testing, treatment or follow-up for such cases. From that perspective, none of these clinical features or combinations of features is very acceptable. It must also be remembered that the positive predictive value for a clinical feature will depend on the prevalence of that feature.[9] In field populations in Nepal, where only 8% of cases classified as MB are HSP, the positive predictive value of these clinical features will be much poorer than in this study.

One needs to look carefully at what action could be taken even if it were possible to identify HSP cases. The Institute Marchoux group recommended that MB cases with an initial average BI $\geq 4{\cdot}0$ receive at least 4 years of standard WHO MDT;[5] however, to our knowledge the efficacy of this treatment has not yet been tested. As yet, no clear international guidelines have been developed regarding the optimal duration of treatment for such cases. Further studies should be undertaken to clarify this matter urgently.

The cost of treating either all or a targeted sub-population of MB cases with MDT for a prolonged period or carefully following the same sort of patients for a decade seems high initially, but may not be excessive. If relapse among the 'highly smear positive' cases proves as frequent as was found by the Marchoux group (39%), and HSP cases are as common as the Nepal survey estimated, 31 MB cases would need to be treated with 'prolonged' MDT or followed up for 8–10 years to prevent one 'highly smear positive' case from relapsing. This needs to be weighed against the lower costs of re-treatment of the relapses with a second course of MDT, assuming that there is no development of drug resistant disease nor the development of additional disability in relapse cases. Until a large cohort of MB cases, who have received only 12 months of MB MDT, have been followed after release from treatment for 7–10 years, it will be impossible to assess the scale of the relapse rate among HSP patients. Whatever decisions are ultimately made regarding the management of HSP cases, control programmes must weigh the social and medical consequences for individual cases who may relapse against the significant logistical and financial obstacles that will have to be overcome by the programme to ensure that all such cases are treated adequately.

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References

[1] WHO Expert Committee on Leprosy, Seventh Report. WHO Technical Report Series, No. 874. World Health Organisation, Geneva, 1998.
[2] Leprosy Unit, Division of Control of Tropical Diseases. *Risk of relapse in leprosy.* WHO/CTD/LEP/94.1. World Health Organisation, Geneva, 1994.
[3] Ganapati R, Revankar CR, Pai W, Girija D. Fixed duration multidrug therapy in leprosy—a preliminary follow-up report. 15th International Leprosy Congress, Beijing (abstract CH37).
[4] Ji B. Why multidrug therapy for multibacillary leprosy can be shortened to 12 months. *Lepr Rev*, 1998; **69**: 106–109.
[5] Jamet P, Ji B, and the Marchoux Chemotherapy Study Group. Relapse after long-term follow-up of multibacillary patients treated by WHO multidrug regimen. *Int J Lepr* 1995; **63**: 195–201.
[6] Marchoux Chemotherapy Study Group. Relapses in multibacillary leprosy patients after stopping treatment with rifampin-containing combined regimens. *Int J Lepr* 1992; **60**: 525–535.
[7] Waters MFR. Relapse following various types of multidrug therapy in multibacillary leprosy. *Lepr Rev* 1995; **66**: 1–9.
[8] Manandhar R, LeMaster J, Roche P. Risk factors for erythema nodosum leprosum. *Int J Lepr* 1999; **67**: 270–278.
[9] Henneken CH, Buring JE. *Epidemiology in medicine*. Little, Brown and Co., Boston, USA, 1987, pp 337–339.

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Mast cells in leprosy skin lesions

VIJAYA V. MYSOREKAR,
CHITRALEKHA P. DANDEKAR & SARASWATI G. RAO
Department of Pathology, M. S. Ramaiah Medical College, Bangalore, India

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Summary The density and distribution of mast cells was assessed in skin biopsies of 118 untreated leprosy cases and 20 healthy individuals taken as controls. Mast cells were present in only small numbers in the skin biopsies of healthy individuals. Significantly higher mast cell counts were obtained in the skin lesions of indeterminate leprosy ($P < 0{\cdot}01$). The mast cell count in the tuberculoid group was significantly lower than that in the lepromatous group ($P < 0{\cdot}05$). The lepromatous group also showed increased mast cell degranulation and altered morphology. The mast cell distribution in the skin biopsies of the two groups was, however, similar. The mast cell count in leprosy is probably determined by the pattern of cytokines released by the T lymphocytes. However, the influence of mast cells on the outcome of the disease needs to be evaluated further.

Introduction

In leprosy, the level of the host cellular immune response to *Mycobacterium leprae* determines the pattern of the disease, thus giving rise to a spectrum of clinical and histopathological features.[1,2] Mast cells have not received much attention in leprosy, but evidence linking them with the development of delayed hypersensitivity reactions[3] raises the possibility that they might be of some significance in leprosy lesions.

The aim of the present study was to assess the density and distribution of mast cells and their possible role in the skin lesions of leprosy.

Materials and methods

The present study was conducted on 118 untreated leprosy patients visiting the Dermatology department of M. S. Ramaiah Medical Teaching Hospital, Bangalore, India. The cases

Correspondence to: V. V. Mysorekar, 55-55A, A.E.C.S. Layout, 60 ft rd, behind ISRO, R.M.V. Extension Stage II, Bangalore 560 094, India (e-mail: dandekar@bgl.vsnl.net.in)

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included 64 males and 54 females ranging in age from 7 to 82 years. In each case, a 4 mm punch biopsy specimen was obtained from the skin lesion, under local anaesthesia with 1% lignocaine. Biopsies taken from the normal skin of 20 healthy individuals undergoing surgery for trauma, served as controls.

Each biopsy was fixed in 10% buffered formalin and sent to the Department of Pathology, M. S. Ramaiah Medical College, Bangalore, India, for processing in the conventional manner. From each skin biopsy, serial paraffin sections of 5 micron thickness were cut and stained with haematoxylin and eosin (H & E), Fite-Faraco stain for acid-fast bacilli and 1% aqueous toluidine blue for mast cells.

The H & E and Fite-Faraco stained sections were studied and the leprosy cases were classified into five groups: lepromatous leprosy (LL), borderline lepromatous leprosy (BL), mid-borderline leprosy (BB), borderline tuberculoid leprosy (BT) and tuberculoid leprosy (TT).[1,2] Cases where there were one or two small hypopigmented macules on the skin with a slight sensory impairment clinically, and where the skin biopsy histologically showed a non-specific lymphocytic infiltration around dermal nerve twigs with occasional acid-fast bacilli, were labelled as indeterminate leprosy. The toluidine blue stained sections were studied for the density and distribution of mast cells. The mast cell counts were performed using a light microscope at $\times 400$ magnification. The average counts obtained in 20 non-overlapping high power fields (HPFs) were considered in each case.

The density of mast cells was expressed as the number of cells per mm^2. The histopathological diagnoses and mast cell counts in all the cases were done independently by all the three authors.

Results

Out of the 118 leprosy cases in the present study, there were 18 LL, 20 BL, 14 BB, 20 BT and 21 TT cases. The remaining 25 cases were diagnosed as indeterminate leprosy. Four of the 18 cases of LL had features of histoid leprosy.

The mean density of mast cells in the 20 control skin biopsies was 12·6 per mm^2. The density of mast cells in the skin lesions in the 118 leprosy cases is given in Table 1. When the results were statistically analysed by using the 'analysis of variance' method, it was found

Table 1. Density of mast cells in the skin lesions of the various types of leprosy

		Mast cell density per mm^2	
Type of leprosy	Number of cases	Mean	Standard deviation (SD)
LL	18	115·3	45·6
BL	20	104·3	32·2
BB	14	68·3	38·9
BT	20	23·4	24·9
TT	21	15·8	20·5
Indeterminate	25	109·0	33·4
Total	118	–	–
Controls	20	12·6	9·4

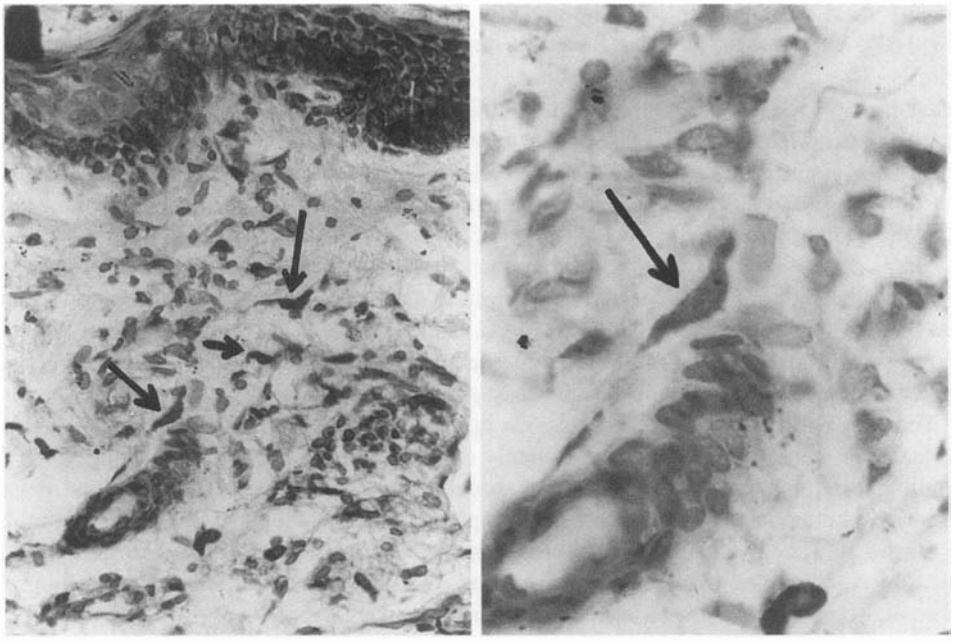

Figure 1. Lepromatous leprosy: subepidermal thin and elongated mast cells (arrows). (a) Toluidine blue ×320; (b) toluidine blue ×800.

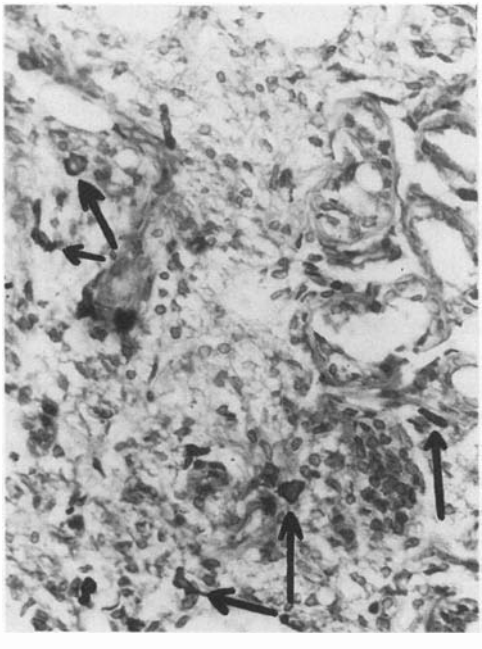

Figure 2. Lepromatous leprosy: mast cells (arrows) are seen amidst the foamy macrophages and around the sweat glands visible in the upper part. Toluidine blue ×320.

that the mast cell density was significantly higher in lesions of indeterminate leprosy ($P < 0{\cdot}01$), LL ($P < 0{\cdot}01$), BL ($P < 0{\cdot}01$) and BB ($P < 0{\cdot}05$) when compared with the controls. The mast cell density in lesions of BT and TT did not differ significantly from the controls ($P > 0{\cdot}05$). As can be seen from Table 1, the mast cell density was found to be decreasing from the lepromatous to the tuberculoid end of the spectrum. There was no statistically significant difference between the mast cell density in LL and BL biopsies ($P > 0{\cdot}05$). Similarly, there was also no statistically significant difference between the mast cell density in BT and TT biopsies ($P > 0{\cdot}05$). Among the two basic groups, tuberculoid (including 21 TT and 20 BT) and lepromatous (including 18 LL and 20 BL), the mast cell density in the first group was found to be significantly lower than that in the second group ($P < 0{\cdot}05$). Three of the 20 BT biopsies (15·0%) and 10 of the 21 TT biopsies (47·6%) studied did not show any mast cells in the sections scanned for these cells. Histoid leprosy biopsies showed mast cell counts similar to other cases of the lepromatous group.

The distribution of mast cells in the skin biopsies of the two groups of leprosy cases was similar. Mast cells were seen subepidermally (Figure 1), in the centre and periphery of the granuloma, in the intervening dermis, and around skin appendages (Figure 2), blood vessels and nerves. In the majority of the cases, macrophages and lymphocytes were seen adjacent to the mast cells. The mast cells in the skin biopsies of LL cases showed altered morphology, appearing thin and elongated (Figure 1) and in different stages of degranulation. An occasional case of LL showed mast cells even in the deeper fat.

Discussion

The shape of the mast cell seems to correspond with its dynamic functional state when fixed.[4] Round to oval mast cells are seen in the normal dermis but following a stimulus, the cell changes its shape and elongates to discharge the granules. Thus, a high mast cell count with mast cell elongation and extensive degranulation in LL indicates their greater functional activity in these lesions.[5]

A predominance of mast cells in the lepromatous group as compared to the tuberculoid group has been described by other authors also,[6,7] although their counts are not quantitative. The present study did not show any difference between the mast cell numbers in histoid leprosy skin biopsies and those from other cases of the lepromatous group, as has also been noted by others.[7]

Mast cells are activated by cytokines including interleukin-3 (IL-3), IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) derived from the T lymphocytes, of which IL-4 is the most essential.[8] The difference in the mast cell counts in the lepromatous and tuberculoid groups has been explained on the basis of the difference in the cytokine patterns and T cell subsets in the two groups.[9,10] In lepromatous lesions, the activated CD 4+ helper T cells of T_H2 type produce high levels of IL-4, which in turn activates the mast cells.[9] On the other hand, in tuberculoid leprosy, the CD 4+ helper T cells are of the T_H1 type. These cells produce undetectable levels of IL-4,[10] thus accounting for the low mast cell response in tuberculoid leprosy. It has been stated that a reduction of mast cells may occur only if the lepromatous lesions upgrade to become more of the tuberculoid type, with changes in the cytokine pattern.[11]

Aroni *et al.*[7] suggest that the larger number of mast cells in the lepromatous group as compared to the tuberculoid group may be linked to the increased vascularity and changes

observed in the endothelial cells which are more obvious in lepromatous leprosy. Mast cell mediators like histamine, heparin and a tumour necrosis factor α (TNF-α)-like molecule are known to be mitogenic for fibroblasts and endothelial cells.[12] Thus, mast cells may also play a role in fibroblastic proliferation following reactive episodes in leprosy.[6]

Cree *et al.*,[13] in their study, found that the granuloma mast cell density was decreased in multibacillary patients as compared to paucibacillary patients. They explain that this might be an apparent decrease due to the increase in dermal volume as a result of oedema in multibacillary patients.

A similar pattern of mast cell distribution has been found in the skin biopsies of the lepromatous and tuberculoid leprosy groups in the present study as well as in other studies.[7,13] It has been suggested that the mast cells in the Grenz zone in LL cases might be related to various cytokines released from the keratinocytes.[14]

A high mast cell count in indeterminate leprosy biopsies was a striking feature in the present study. This observation has been reported by other authors also.[6,15] Thus, it is likely that a mast cell response occurs in the initial stages of evolution of leprosy.[6] Hence, we feel that a mast cell count on skin biopsies may be of diagnostic value in indeterminate leprosy.

It would have been interesting to know the effect of anti-leprosy treatment on the mast cell counts in the skin lesions in the present cases. However, unfortunately, such a follow-up study could not be conducted in our set-up for two reasons: 1) some patients do not follow strictly the prescribed drug regimen, and hence may show unreliable mast cell counts in follow-up biopsies, and 2) some patients do not come for follow-up as they have to travel from far off villages. However, we suggest that further studies on mast cells in treated leprosy patients would be worthwhile, as they would provide valuable information on the role of immunomodulatory drugs in determining the immune response related to mast cells.

In conclusion, the present study indicates that a prominent mast cell response occurs in the skin lesions of the lepromatous group and indeterminate leprosy. However, the exact significance of mast cell proliferation and degranulation in leprosy is not known. The point whether mediators from the mast cells have any influence on the outcome of the disease needs further evaluation.

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References

[1] Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five group system. *Int J Lepr*, 1966; **34**: 255–273.
[2] Ridley DS. Histological classification and the immunological spectrum of leprosy. *Bull WHO*, 1974; **51**: 451–465.
[3] Galli SJ, Dvorak AM. What do mast cells have to do with delayed hypersensitivity? *Lab Invest*, 1984; **50**: 365–368.
[4] Kumar R. Vaidya MC, Keshwani NH. In vivo response of mast cells to lepromatous serum. *Lepr India*, 1979; **51**: 49–53.
[5] Kumar R. Mast cells in histoid lepromatous lesions. *Ind J Lepr*, 1987; **59**: 390–392.
[6] Rav SD, Pratap VK, Sharma NK, Dayal SS. Mast cell in leprosy. *Ind J Lepr*, 1990; **62**: 467–472.
[7] Aroni K, Kontochristopoulos G, Liossi A, Panteleos D. An investigation of mast cells in two basic leprosy groups. *Int J Lepr*, 1993; **61**: 634–635.

[8] Cotran RS, Kumar V, Collins T. Diseases of immunity. In: Robbins (ed) *Pathologic basis of disease*, 6th edn. Saunders, Philadelphia, 1999, pp 188–259.
[9] Yamamura M, Uyemura K, Deans RJ *et al*. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science*, 1991; **254**: 277–279.
[10] Haanen JBA, De Waal-Malefut R, Res PCM *et al*. Selection of a human T helper type l-like T cell subset by mycobacteria. *J Exp Med*, 1991; **174**: 583–592.
[11] Jayalakshmi P. Mast cells in lepromatous leprosy. *Int J Lepr*, 1995; **63**: 291–293.
[12] Rothe MJ, Kerdel FA. The mast cell in fibrosis. *Int J Dermatol*, 1991; **30**: 13–16.
[13] Cree IA, Coghill G, Swanson Beck J. Mast cells in leprosy skin lesions. *J Clin Pathol*, 1990; **43**: 196–200.
[14] Kupper SK. Mechanisms of cutaneous inflammation. *Arch Dermatol*, 1989; **125**: 1406–1412.
[15] Liu Zijun *et al*. Histopathology of primary indeterminate leprosy. *Chin J Dermatol*, 1980; **13**: 129 (abstracted in *Int J Lepr*, 1982; **50**: 550).

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Clinical, electrophysiological, and immunopathological study of peripheral nerves in Hansen's disease

WAFAA RAMADAN,* BASMA MOURAD,*
WAEL FADEL,** & ENAS GHORABA*
*Departments of *Dermatology and **Neurology, Faculty of Medicine, Tanta University, Tanta, Egypt*

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Summary Hansen's disease is a disease of peripheral nerves. Some patients develop peripheral neuropathy before the diagnosis of the disease, and others develop these complications after starting therapy. Electrophysiological (EP) studies were carried out in Hansen's disease patients. This work studied the neural deficits, electromyography (EMG) and motor nerve conduction (MNC) variables in different types of leprosy and the immunopathology of sural nerve tissue in patients with severe neural deficits. Forty leprosy patients had neurological examinations and EP study. Histopathological and immunopathological study of sural nerve biopsy specimens was performed for 10 patients with severe neural deficits. The results of the neurological study showed that there was involvement of cranial nerves, muscular system, motor reflexes and sensory system and trophic and vasomotor changes. EP study showed significant changes in EMG of abductor digiti minimi in patients as compared to controls. MNC variables of common peroneal nerve were abnormal in 80% of all patients, MNC of median nerve was abnormal in 72.5%, while MNC of ulnar nerve was abnormal in 70% and SNC of ulnar nerve was abnormal in 77.5% of the total. In conclusion, electrophysiological investigations have an important role in the detection of muscle denervation and neuropathic changes in leprosy patients. These investigations are safe, rapid and non-invasive techniques. On the other hand immunopathological study revealed that the degree of immune positivity correlated with the degree of nerve fibrosis.

Introduction

Although its overall prevalence is decreasing, leprosy continues to be a major cause of neuropathy worldwide,[1] as *Mycobacterium leprae* has the capacity to invade the peripheral nervous system and cause neuropathy.[2] Leprous neuropathy is characterized by involvement of dermal nerves and superficial peripheral nerve trunks in cooler body regions.[1] There are four possible mechanisms of nerve damage in all forms of HD: (1) the presence of *M. leprae* or its antigens at cooler sites; (2) trauma of superficially placed nerve trunks; (3) increased

Correspondence to: W. Ramadan

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intraneural pressure; and (4) vascular changes in intraneural blood vessels.[3] HD leads to change in muscle function resulting in muscle imbalances with deformity of soft tissues and joints.[4] The precise pathophysiological mechanism of peripheral neuropathy is not yet clear. It is not due to the invasion of nerves by living bacilli but it may be due to a later immune response from antigen-antibody reaction to dead bacilli and the peripheral nerves become fibrosed from mechanical stress.[4]

AIM OF THE WORK

This work studied the neural deficits and electrophysiological changes in different types of leprosy and the immunopathological status of the neural tissue in patients with severe neural deficits, to increase our insight into the pathophysiology of neural involvement in leprosy.

Materials and methods

Forty treated (received MDT) leprosy patients (5 PN, 2 TT, 16 BT, 2 BL, and 15 LL) and 10 normal healthy persons (matching the same age and sex of the patients) serving as control were the subject of this study. The patients were 26 males and 14 females and their age ranged from 19 to 80 years with a mean value of 44·9 ± 14·57. The duration of the disease ranged from 6 months to 40 years. Eighteen of these patients had experienced leprosy reactions (7 LL, 10 BT and 1 BL).

METHODS

All patients and controls had complete history taking, thorough clinical and dermatological examinations, and full neurological examinations: examination of cranial nerves, peripheral nerves, motor system (muscles and motor reflexes) and sensory system (superficial and deep sensations).

Cranial nerve assessment

This was carried out by the detection of clinical manifestations of nerve impairment, e.g. optic nerve (distributed visual acuity, and colour vision), trigeminal nerve (hypothesia of the face, wasting of pterygoid, masseter and temporalis muscles and decreased or lost corneal reflex), facial nerve (lower motor neuron facial paralysis), cochleovestibular nerve (decreased acuity of hearing), glossopharyngeal nerve (decreased pharyngeal reflex) and vagus nerve (hoarseness of voice and dysphagia).

Examination of specific peripheral nerves

The ulnar, median and common peroneal nerves were palpated at the elbow, wrist and knee respectively.[5]

Muscle power assessment

This was performed by testing the contraction of the muscle against gravity and resistance.

Sensory system assessment

Superficial sensory system was assessed by using the pin prick test for pain sensation, hot test tube for temperature and a piece of thin paper, a feather or a wisp of cotton-wool for light touch sensation in both upper and lower limbs. Testing joint, vibration and pressure sensations assessed the deep sensory system.

INVESTIGATIONS

- Slit skin smear for all patients for the confirmation of diagnosis and classification of patients.
- Electrophysiological study including:

  Needle EMG: The detection and recording of the electrical activity from a portion of a muscle (recording of motor unit potentials). The apparatus used was the Dantec - Neuromatic 2000 C.2-channel. The muscles examined were the abductor pollicis brevis for testing the integrity of the median nerve, abductor digiti minimi for testing the ulnar nerve and extensor digitorum brevis for testing the common peroneal nerve. For each muscle EMG was done in three phases: during insertion of needle, at rest and at full volition.[6]

  Normal EMG: Complete electric silence at rest. With minimal voluntary contraction, individual motor unit potentials are seen, this represents the summation of membrane action potentials of many muscle fibres, which innervated by the same anterior horn cell (the motor unit). With increasing contraction, more units are recruited and the firing rate increases. Since individual motor units cannot be distinguished, this is referred to as a complete interference pattern.[6]

  Abnormal EMG: Changes with neuropathy: these include, fibrillation, fasciculation, giant motor unit potentials and reduced interference or recruitment pattern.[6]

  Fibrillation: the contraction of a single muscle fibre, which appears when the muscle fibre has lost its nerve supply.[6]

  Fasciculation: the spontaneous or involuntary contraction of a motor unit or a small group of motor units. The waveforms are bizarre and irregular, occurring most often in motor neuron disease and may occur in normal persons after exhausted exercise or with general fatigue.[6]

  Giant motor unit potentials: occur in chronic neuropathy, where there is incomplete denervation. The surviving axons from sprouts, which reinnervate neighbouring denervated muscle fibre, leads to enlargement of the motor units.[6]

  Reduced interference pattern: the reduction in the number of motor units in a denervated muscle diminishes the interference pattern on voluntary muscle contraction.[6]

  Changes with myopathy: motor unit potential is lower in amplitude and shorter in duration than normal, and there is reduced interference pattern.[6]

  Motor nerve conduction (MNC) variables: These include motor nerve conduction velocity (MCV), distal latency (DL), amplitude (amp) and stimulus strength (st.st). The apparatus used for EMG measures them.[6]

  MCV: recording muscle action potential from the stimulation of motor nerves.[6]

  DL: the time from the onset of stimulus artifact to the onset of response.[6]

  St.st.: measures the strength of muscle contraction due to electric stimulation of

motor nerves. The stimulating electrodes are placed on the left end of the nerve, and the recording leads are on the right end.

Figure 1 illustrates the recording situation. Stimulation is started gradually. At the beginning there is no action potential, because the stimulus is subthreshold. Increasing the stimulus strength elicits the appearance of a small action potential when the nerve threshold is reached. By still further increasing the intensity of stimulation, the action potential of the nerve begins to grow in amplitude, up to a maximum. This is the maximal response, evoked by the maximal stimulation. Points between threshold and maximal stimulation are called either suprathreshold or submaximal. Stimulating with weak currents, activates the most excitable fibres in the nerve, with increasing the stimulus strength, fibres of lower excitability or higher threshold begin to recruit.[7]

Sensory nerve conduction (SNC) variables of ulnar nerve only: These include sensory conduction velocity (SCV), DL, amp., and st.st. This was done in a similar manner to MNC.[6]

SCV: recording nerve action potential from the stimulation of sensory nerves.[6]

- Histopathological study:
  Nerve biopsy specimens of sural nerve were taken from 10 patients with severe neurological deficits (diagnosed clinically by detecting the impairment of motor, sensory and/or autonomic nerve function and by EP to detect the extent of nerve impairment). The age of these patients ranged from 50 to 80 years and the duration of the disease ranged from 15 to 40 years. The nerve biopsy specimen was taken from the most affected limb. Each specimen was divided into two halves, one half for histopathological study [stained with H&E stain and Mallory's phosphotungistic acid haematoxylin (PTAH) stain],[8] and the other half for immunopathological study.
- Immunopathological study:
  Streptavidin–biotin immunoperoxidase (ABIP) staining and Immustain Polyclonal Rabbit Primary Antibody set for IgG and IgM (DPC Diagnostic Products Corporation).[9]

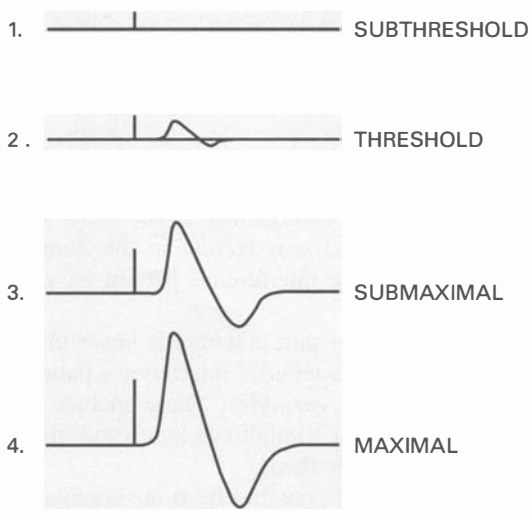

Figure 1. Effects of different stimulus strength on the amplitude of the action potential during stimulation of a whole nerve.

Method[9]

1. Prepare buffered wash working solution.
2. Prepare duplicate slides for each specimen.
3. Removal of paraffin and rehydration of sections.
4. Place blocking endogenous peroxidase on each slide.
5. Trypsinization of tissue sections in 0.1 trypsin solution.
6. Exposure to primary antibody B1 Immustain Polyclonal Rabbit Primary Antibody set for IgG and DPC's Immustain Polyclonal Rabbit antibody set for IgM.
7. Exposure to polyclonal linking reagent.
8. Exposure for streptavidin enzyme.
9. Colour development: put several drops of DI-amino-benzidine working colour reagent on each slide, then counter stain with Mayer's haematoxylin.
10. Interpretation of the results: view the slides on bright-field microscope. The intensity of staining was graded as follows: + (mild), ++ (moderate) and +++ (marked).

Results

CLINICAL RESULTS

Table I shows the distribution of leprosy patients according to age, duration of the disease and type of leprosy.

NEUROLOGICAL RESULTS

Table 2 shows leprosy patients with impairment of:

- Cranial nerves: the trigeminal nerve was the most common cranial nerve impaired, being involved in 19 patients (47·5%). The predominant manifestations of trigeminal nerve

Table 1. Distribution of leprosy patients studied according to age, duration of the disease, and type of leprosy

	Types of leprosy patients ($n = 40$)					
	LL ($n = 15$)	BL ($n = 2$)	BT ($n = 16$)	TT ($n = 2$)	PN ($n = 5$)	Total ($n = 40$)
Age/years						
<20	–	1	–	–	1	2
20–40	3	1	4	2	4	14
41–60	10	–	9	–	–	19
71–80	4	–	1	–	–	5
Duration/years						
<1	–	1	–	–	2	3
1–5	4	–	2	1	3	10
6–10	2	1	5	1	–	9
11–15	1	–	4	–	–	5
16–20	3	–	1	–	–	4
>20	5	–	4	–	–	9

affection were mainly sensory manifestations in the form of hypothesia of the face in 12 patients, anaesthesia of the face in seven patients and loss of corneal reflex in all of them. Motor manifestations were detected in 10 patients only (wasting and weakness of temporalis and masseter muscles in seven patients, and deviation of the lower jaw due to the affection of pterygoid muscle in three patients).

- Muscular system: the commonest disabilities in muscles were manifested by: claw hand(s) in 13 patients (32·5%), wasting of hand(s) and/or forearm(s) in 31 patients (77·5%) [unilateral distal 1/3 wasting towards ulnar side (flexors of wrist, long flexors of 4th and 5th digits and intrinsic hand muscles) in 10 cases, distal 1/3 wasting towards radial side (triceps, brachioradialis, wrist, finger and thumb extensors) in three cases, unilateral wasting of hypothenar muscles in four cases, bilateral distal 2/3 wasting in five cases and bilateral distal 1/3 wasting in nine cases] and lower limb(s) wasting in 21 patients (52·5%) [wasting of distal leg muscles towards peroneal side in four cases, wasting of foot muscles in three cases, bilateral wasting of distal 1/3 of legs in three cases and bilateral distal 2/3 wasting of legs in 11 cases], hypotonia of upper and lower limbs in 18 patients (45%) and 13 patients (32·5%), respectively, and decreased muscle power of upper and lower limbs in 12 patients (30%) and 11 patients (27·5%), respectively.
- Motor reflexes: supinator reflex was the most affected, diminished in 18 patients (45%); least affected were triceps and knee reflexes, each diminished in seven patients (17·5%).
- Sensory system: superficial and deep sensations were affected as shown.
- Trophic and vasomotor changes: the most common manifestations were resorption of fingers in 18 patients (45%), anhidrosis in 17 patients (42·5%) and resorption of toes in 13 patients (32·5%).

With regard to nerve thickness, thickened ulnar nerve was found in 17 patients, thickened median nerve in five patients, thickened radial nerve in two patients, thickened great auricular nerve in nine patients and thickened common peroneal nerve in 10 patients.

ELECTROPHYSIOLOGICAL RESULTS

EMG results

Table 3 shows EMG results of some muscles in leprosy patients. There was abnormal EMG of abductor pollicis brevis in 29 patients (two had fibrillation potentials, 27 had reduced or discrete recruitment pattern), abnormal EMG of abductor digiti minimi in 28 patients (four had fibrillation potentials, 24 had reduced or discrete recruitment pattern) and abnormal EMG of extensor digitorum brevis in 24 patients (two had fibrillation potentials, 22 had reduced or discrete recruitment pattern).

Table 4 shows statistical analysis of EMG amplitude of some muscles in leprosy patients and controls. There was a non-significant reduction in EMG amp. of abductor pollicis brevis in patients as compared to controls, significant reduction in EMG amp. of abductor digiti minimi and non-significant reduction in EMG amp. of extensor digitorum brevis.

Nerve conduction results

Tables 5, 6 and 7 show statistical analysis of MNC variables of median nerve, ulnar nerve (Figure 2) and common peroneal nerve, respectively. There was a statistically significant

Table 2. Neurological manifestations in different types of leprosy patients studied

Neurological manifestations	Types of leprosy patients ($n = 40$)					
	LL	BL	BT	TT	PN	Total
Cranial nerves affected:						
Optical nerve	7	1	–	–	–	8
Trigeminal nerve	17	1	–	1	–	19
Facial nerve	–	–	5	2	–	7
Glossopharyngeal	3	–	3	–	–	6
Vagus	7	–	6	–	–	13
Cochleovestibular	3	1	1	–	–	5
Muscular system:						
Disability:						
Claw hand	3	–	7	1	2	13
Hand drop	1	–	–	–	–	1
Flexion deformity (toes)	2	–	–	–	1	3
Muscle wasting of:						
Upper limb(s)	12	1	13	2	3	31
Lower limb(s)	12	–	5	1	3	21
Hypotonia of:						
Upper limb(s)	6	11	1	–	–	18
Lower limb(s)	7	–	4	–	2	13
Decreased muscle power of:						
Upper limb(s)	5	7	–	–	–	12
Lower limb(s)	7	4	–	–	–	11
Motor reflexes:						
Diminished						
Biceps reflex	5	–	3	–	–	8
Supinator reflex	7	–	10	1	–	18
Triceps reflex	4	–	3	–	–	7
Knee reflex	5	–	2	–	–	7
Ankle reflex	6	–	2	–	2	10
Loss of:						
Ankle reflex	4	–	2	–	–	6
Sensory system:						
Superficial sensation:						
Mononeuropathy on:						
Ulnar side	3	1	7	1	1	13
Radial side	1	–	–	1	–	2
Peroneal side	4	–	1	–	1	6
Glove and stocking hypothesia	8	–	6	–	1	15
Glove hypothesia	–	1	1	–	1	3
Stocking hypothesia	2	–	1	–	–	3
Maculoanaesthetic patches						
Deep sensation:						
Diminished joint & vibration sensation	8	–	5	–	–	13
Trophic & vasomotor changes:						
Resorption of fingers	8	–	10	–	–	18
Resorption of toes	9	–	4	–	–	13
Fissuring foot	3	–	6	1	3	13
Plantar ulceration	3	–	–	–	–	3
Joint deformity	4	–	2	–	–	6
Osteoarthritis	2	–	3	–	–	5
Anhidrosis	5	–	8	1	3	17

Table 3. EMG results of some muscles in leprosy patients

	Patients ($n = 40$)		
		Abnormal	
EMG of	Normal	Fibrillation potentials	Reduced or discrete recruitment pattern
Abductor pollicis brevis	11	2	27
Abductor digiti minimi	12	4	24
Extensor digitorum brevis	16	2	22

Table 4. Statistical analysis of EMG amplitude of some muscles in leprosy patients and controls. mv = millivolt

	EMG amplitude of					
	Abductor pollicis brevis		Abductor digiti minimi		Extensor digitorum brevis	
	Patients $n = 40$	Control $n = 10$	Patients $n = 40$	Control $n = 10$	Patients $n = 40$	Control $n = 10$
Range	0·3–2·1 mv	0·5–2·1 mv	0·2–1·8 mv	0·8–2·3 mv	0·32–2·0 mv	0·5–0·2 mv
Mean	1·11	1·25	0·91	1·59	1·09	1·25
±SD	0·58	0·53	0·67	0·49	0·54	0·57
T	0·71		3·63		0·86	
P	0·05		0·01*		0·05	

* Significant.

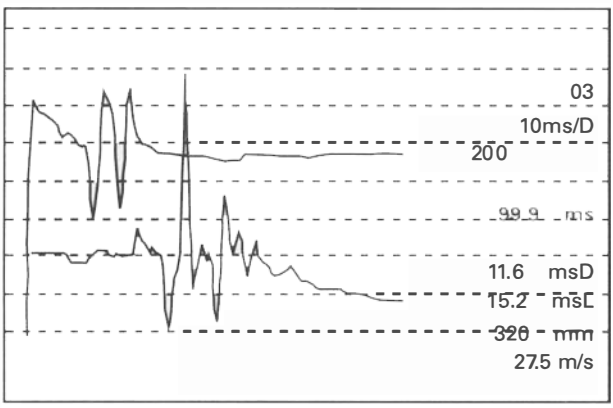

Figure 2. Motor nerve conduction study of ulnar nerve in a patient with LL showing decreased NCV and Amp. with increased DL and St. st.

reduction in MCV, significant prolongation of DL, and significant reduction of amp. of median nerve in 72.5% of patients, of ulnar nerve in 70% and of common peroneal nerve in 80% of patients. There was a significant increase of St. st of all previous nerves in 100% of patients.

Table 8 shows statistical analysis of SNC variables of ulnar nerve. There was a significant

Table 5. Statistical analysis of motor nerve conduction variables of median nerve in leprosy patients and controls. ms = millisecond, m/s = meter/second, μv = microvolt

	Patients ($n = 40$)			Controls ($n = 10$)				
Median motor nerve variables	Range	Mean	±SD	Range	Mean	±SD	*t*	*P*
CV m/s	0–51·6	31·11	17·33	48·2–58·2	52·01	3·03	7·20	<0·001*
D.L. ms	0–15·6	7·23	4·75	3·04–4·22	3·91	0·38	4·37	<0·001*
Amp mv	0–10	6·94	3·22	11·5–15	13·05	1·04	10·09	<0·001*
St. st. μv	37–99·9	90·82	21·26	8·13	10·50	1·96	23·50	<0·001*

*Significant.

Table 6. Statistical analysis of motor nerve conduction variables of ulnar nerve in leprosy patients and controls

	Patients $n = 40$			Controls $n = 10$				
Ulnar motor nerve variable	Range	Mean	±SD	Range	Mean	±SD	*t*	*P*
CV m/s	0–50	26·14	17·65	51·6–66·9	58·13	4·88	10·03	<0·001*
D.L. ms	0–18	8·29	5·97	2·36–3·12	2·79	0·23	5·82	<0·001*
Amp mv	0–44	3·30	7·01	11·3–15	13·02	1·27	4·34	<0·001*
St. St. μv	80–99·9	97·91	6·05	8·13	11·10	1·91	44·54	<0·001*

* Significant.

Table 7. Statistical analysis of motor nerve conduction variables of common peroneal nerve in leprosy patients and controls

	Patients $n = 40$			Controls $n = 10$				
Common peroneal motor nerve variable	Range	Mean	±SD	Range	Mean	±SD	*t*	*P*
CV m/s	0–41.3	28·27	13·98	45·4–51·8	47·84	2·29	8·42	<0·001*
D.L. ms	0–15.2	7·77	4·85	3·76–5·44	4·63	0·63	3·96	<0·001*
Amp mv	0–4	2·29	1·35	9·5–15	12·48	1·99	15·32	<0·001*
St. St. μv	76–99·9	96·23	8·10	9–13	11·00	1·76	61·04	<0·001*

* Significant.

Table 8. Statistical analysis of sensory nerve conduction variables of ulnar nerve in leprosy patients and controls

	Patients $n = 40$			Controls $n = 10$				
Ulnar sensory nerve variable	Range	Mean	±SD	Range	Mean	±SD	*t*	*P*
CV m/s	0–36·6	16·61	14·25	47·3–54·2	49·92	2·65	13·86	<0·001*
D.L. ms	0–15	7·87	5·56	2·2–3·08	2·68	0·30	5·87	<0·001*
Amp mv	0–60	24·13	21·00	17–100	42·13	30·77	2·20	<0·001*
St. St.μv	40–93·5	47·25	8·38	8·8–12·4	10·08	1·27	13·88	<0·001*

* Significant.

Table 9. Evaluation of MNC variables of different nerves and SNC variables of ulnar nerve

	Nerve conduction variables							
	Normal		Abnormal					
			Conducting Nerve		Non-conducting nerve		Total	
Nerve	*n*	%	*n*	%	*n*	%	*n*	%
Median	11	27·5	23	57·5	6	15·0	29	72·5
Ulnar (motor)	12	30·0	20	50·0	8	20·0	28	70·0
Common peroneal	8	22·0	28	60·0	4	10·0	32	80·0
Ulnar (sensory)	9	22·5	19	47·5	12	30·0	31	77·5

reduction of SCV, significant prolongation of DL, significant reduction of amp. in 77.5% of patients and significant increase of st. st. in 100% of patients.

Table 9 shows that MNC of median nerve was abnormal in 29 patients (72.5%) (the nerve was non-conducting in six patients). MNC of ulnar nerve was abnormal in 28 patients (70%) of the total (non-conducting nerve in eight patients) and MNC of common peroneal was abnormal in 32 patients (80%) of the total (non-conducting nerve in four patients). SNC of ulnar nerve was abnormal in 31 patients (77.5%) (non-conducting nerve in 12 patients), while st. st. was abnormal in all patients.

HISTOPATHOLOGICAL RESULTS

Table 10 shows the distribution of the patients who were included in the histopathological study according to age, duration of the disease and type of leprosy.

Table 11 shows the histopathological changes of sural nerve biopsy specimens in leprosy patients by using different stains.

Table 10. Distribution of leprosy patients who were used for histopathological study according to age, duration of disease and type of disease

	Patients ($n = 10$)		
	LL	BT	Total ($n = 10$)
Age (years)			
50–60	4	2	6
61–70	1	2	3
>70	1	–	1
Duration (years)			
20–25	3	2	5
26–30	2	2	4
>30	1	–	1

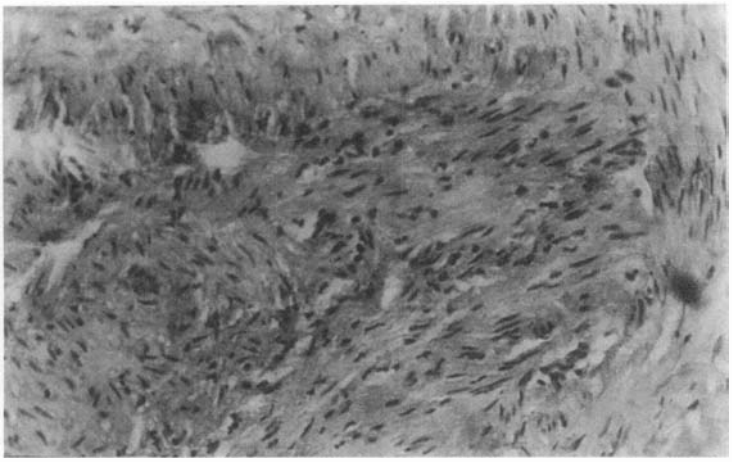

Figure 3. Marked nerve fibrosis replacing the endoneural tissue in BT (H&E×125).

H&E STAIN

There was fibrosis of the intraneural tissue, [marked fibrosis of endoneural tissue in two patients (20%) out of 10 (Figure 3), moderate in six patients (60%) and mild in two patients (20%)], destruction of the perineurium, areas of breakdown of myelin sheath, degenerative changes of Schwann cells and no evidence of invading inflammatory cell infiltrate in sural nerve biopsy specimens in most patients (Table 11).

Table 11. Histopathological changes of sural nerve biopsy specimens in leprosy patients by using different stains

	Leprosy patients ($n = 10$)	
Histopathological changes	LL ($n = 6$)	BT ($n = 4$)
H & E:		
Mild fibrosis	–	2
Moderate fibrosis	4	2
Severe fibrosis	2	–
Mallory's PTAH:		
Fibrosis	6	–
No fibrosis	–	4
ABIP:		
IgG deposition		
+	–	2
++	4	2
+++	2	–
IgM deposition		
+	–	2
++	1	2
+++	5	–

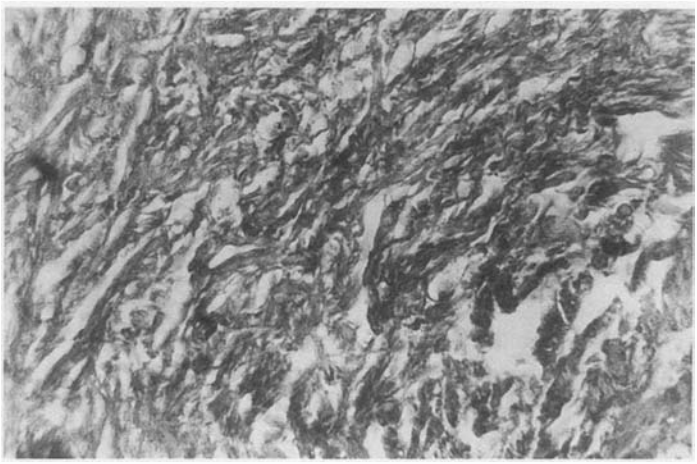

Figure 4. Extensive nerve fibrosis with scattered scanty myelinated nerve fibres in BT (Mallory's × 125).

HISTOCHEMICAL STAIN (MALLORY'S PTAH)

There was predominant fibrosis of the nerve trunk in six cases (60%) out of 10 (Figure 4 and Table 11).

IMMUNOPATHOLOGICAL RESULTS (ABIP STAIN)

There was mild deposition (+) of IgG in two patients (20%), moderate (++) in six patients (60%) (Figure 5), and marked (+ + +) in two patients (20%).

IgM deposition was mild in three patients (30%), moderate in two patients (20%), and marked in five patients (50%).

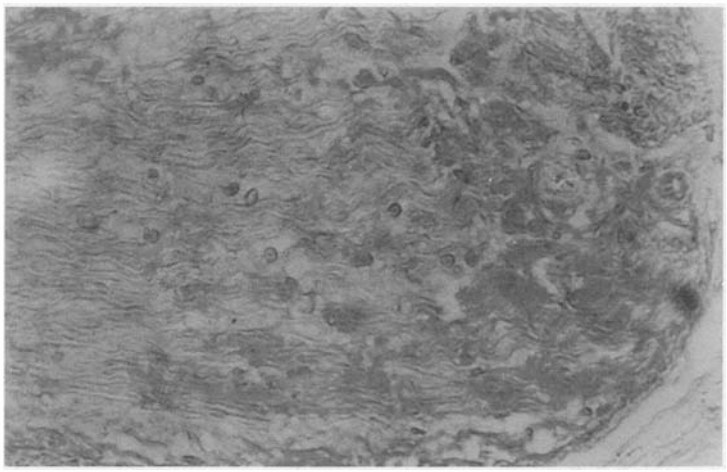

Figure 5. Moderate deposits of IgG (++) in sural nerve section in PN (ABIP × 250).

There were areas of uneven distribution of IgG and IgM in the neural tissue in most specimens. The antigens (IgG and IgM) deposited and appeared as homogenous and reddish patches in the endoneural areas (Table 11).

Discussion

The mechanism of nerve damage in HD remains diverse and unclarified.[10] It may be intrafascicular, intraneural, extrafascicular or extraneural lesions.[11] Peripheral nerve involvement is usually more and appears earlier in TT than in LL and also certain nerves are affected more than others in HD.[12] Croft *et al.*[13] found that the most commonly affected nerve by function impairment was the posterior tibial (sensory) followed by the ulnar nerve.

In this study, the cranial nerves were affected in this order of sequence, the trigeminal followed by optic, then facial, vagus, glossopharyngeal, cochleovestibular and finally spinal accessory nerves. Cranial nerve impairments were more in LL except the facial nerve, which was affected only in BT. This finding was similar to that mentioned by Lubbers *et al.*[14]

Claw hand was the most common disability among patients in the present study; this indicates that ulnar nerve is the most affected nerve in leprosy patients. The impairments were noticed more in LL, and this can be attributed to long duration of the disease, the presence of large number of skin lesions, occurrence of more invasive type and frequent episodes of reaction in those patients. This agrees with the findings of other studies.[15,16]

In the present study, all sensory modalities (superficial and deep) were affected except deep pressure sensation, which was diminished in the advanced cases only. This agrees with the results of Andersen.[5] Trophic and vasomotor changes were manifested in the form of resorption of fingers and/or toes, anhidrosis, fissuring of foot, joint deformity, osteoarthritis and plantar ulceration.

Mshana *et al.*[12] mentioned that some nerves that appeared to be clinically normal have been shown to have pathological changes.

Van Brakel and Khawas[17] recommended that all leprosy patients should have a nerve function assessment at every visit to the clinic at least during their first year of treatment and regular nerve function assessment was essential to detect silent neuropathy at an early stage and to prevent permanent impairment of nerve function.[17]

In the present study EMG results showed a neuropathic pattern, which could be attributed to the sequelae of nerve degeneration rather than muscle degeneration. Thus it can be suggested that the wasting of muscles encountered among leprosy patients with neuropathy may be due to neurogenic atrophy.

Werneck *et al.*[18] stated that the involvement of skeletal striated muscles in leprosy was considered secondary due to peripheral neuropathy, and others attributed it to a primary muscle lesion. In the present study, MNC variables showed that ulnar nerve was more affected than median and common peroneal nerves; this was in agreement with the results of Antia *et al.*[19]

In this study, there was a markedly significant increase of st.st. in all patients even in cases with normal conduction velocity. This means that st. st is a very important and sensitive test for early detection of nerve involvement, which becomes abnormal before any noticeable changes in other motor nerve conduction variables.

Samant *et al.*[20] reported that nerve conduction measurements proved to be more sensitive

in detecting nerve function impairment, but the combination of physical palpation for nerve thickening and graded nylon test was closely comparable to measurement of nerve conduction.

In the present study, SCV of ulnar nerve was slower than MCV, indicating that the sensory system was more affected than the motor system in leprosy patients. This agrees with the findings of other studies.[20,21]

Regarding the histopathological results, there was fibrosis of the intraneural tissues, destruction of the perineurium, areas of breakdown of myelin sheath, degenerative changes of Schwann cells and no evidence of invading inflammatory cell infiltrate in sural nerve biopsy specimens in most patients. Van Brakel and Khawas[22] found nearly similar results.

On the other hand, Mafoyane *et al.*[23] found chronic granulomatous infiltrate within the nerve bundles and extensive destruction and fibrosis of the nerves in patients with primary neuritic leprosy. This variation may be due to the fact that all the studied patients were chosen from those with advanced neural deficits, so fibrosis was the end result of inflammatory changes.

As regards the immunopathological results, in our study, there was marked deposition of IgG and/or IgM in specimens with extensive neural fibrosis. IgG and IgM deposition in the neural tissues may be attributed to the transmission of these deposits through the endoneural blood vessels.

Barros *et al.*[24] found high antigenic load, demonstrated by using anti-BCG antibody (peroxidase-antiperoxidase technique) in the nerve specimens of their patients (in treated LL patients and in untreated TT patients).

In this study, the neural tissues showed areas of unequal distribution of IgG and IgM, this may be attributed to uneven involvement of nerve fascicles or may be related to the chronological occurrence of nerve damage among different nerve fibres or fascicles in the same nerve, this point needs further studies on different nerve biopsy specimens and different grades of nerve damage in a sufficient number of leprosy patients.

References

[1] Nations SP, Katz JS, Lyde CB, Barohn RJ. Leprous neuropathy: an American perspective. *Semin Neurol*, 1998; **18:** 113–124.

[2] Rambukana L. How does *Mycobacterium leprae* target the peripheral nervous system? *Trends Microbiol*, 2000; **8:** 23–28.

[3] Job CK, Nerve damage in Hansen's disease. Part II. *The Star*, 1991; **50:** 5–7.

[4] Krotoski JB. Peripheral neuropathy and examination of the hands. *The Star*, 1991; **50:** 1–5.

[5] Andersen JG. Sensory testing under field conditions. *The Star*, 1990; **50:** 6–7.

[6] Gabr M. *Clinical, biochemical, and electrical study of skeletal muscles in patients with diabetic neuropathy.* MD Thesis, Faculty of Medicine, Tanta University, Tanta, Egypt, 1989.

[7] Eyzaguirre C, Fidone JS. *Physiology of the nervous system. An introductory text. Part 1: general neurophysiology. The nerve.* YearBook Medical Publishers Incorporated Chicago, 1975; pp. 20–30.

[8] Bancroft JD, Stevens A. *Histopathological stains and their diagnostic uses.* Churchill Livingstone, Edinburgh, New York, 1974, pp 3–17.

[9] Taylor CR, Kledisek G. Immunohistologic techniques in surgical pathology – a spectrum of new special stain. *Hum Pathol*, **12:** 590–595.

[10] Antia NH, Shetty VP. Nerve damage in leprosy. *Int J Lepr*, 1988; **56:** 619–621.

[11] Charosky CB, Gatti JC, Cardama JE. Neuropathies in Hansen's disease. *Int J Lepr*, 1983; **51:** 576–586.

[12] Mshana RN, Humber DP, Harboe M, Belehu A. Demonstration of mycobacterial antigens in nerve biopsies from leprosy patients using peroxidase-antiperoxidase immunoenzyme technique. *Clin Immunol Immunopathol*, 1983; **29:** 359–368.

[13] Croft RP, Richardus JH, Nicholls PG, Smith WC. Nerve function impairment in leprosy: design, methodology,

and intake status of a prospective cohort study of 2664 new leprosy cases in Bangladesh (The Bangladesh Acute Nerve Damage Study). *Lepr Rev*, 1999; **70:** 140–159.

[14] Lubbers WJ, Schopper A, Hogeweg M, Soldenhoff R. Paralysis of facial muscles in leprosy patients with lagophthalmos. *Int J Lepr*, 1994; **66:** 220–224.

[15] Smith WCS. The epidemiology of disability in leprosy including risk factors. *Lepr Rev*, 1992; **63:** 23–30.

[16] Chopra NK, Arawal JS. Hansen's disease deformities: an epidemiological study in a multidrug therapy project. *Star*, 1990; **49:** 9–12.

[17] Van Brakel WH, Khawas IB. Silent neuropathy in leprosy: an epidemiological description. *Lepr Rev*, 1994; **65:** 350–360.

[18] Werneck LC, Teive HA, Scola RH. Muscle involvement in leprosy. Study of the anterior tibial muscle in 40 patients. *Arq Neuropsiquiatr Sep.* 1999; **57:** 723–734.

[19] Antia NH, Pandya SS, Dastur DK. Nerves in the arm in leprosy. Clinical, electrodiagnostic and operative aspects. *Int J Lepr*, 1970; **38:** 12–28.

[20] Samant G, Shetty VP, Uplekar MW, Antia NH. Clinical and electrophysiological evaluation of nerve function impairment following cessation of multidrug therapy in leprosy. *Lepr Rev*, 1999; **70:** 10–20.

[21] Vegrhese M, Ittimani KV, Satyanarayan KR. A study of the conduction velocity of the motor fibres of ulnar and median nerves in leprosy. *Int J Lepr*, 1970; **38:** 271–277.

[22] Van Brakel WH, Khawas IB. Nerve damage in leprosy: An epidemiological and clinical study of 396 patients in west Nepal. Part 1. Definitions, methods and frequencies. *Lepr Rev*, 1994; **65:** 204–221.

[23] Mafoyane NA, Lucas SB, Khawas IB. Primary neuritic leprosy in a black South Africa. *Lepr Rev*, 1992; **63:** 277–281.

[24] Barros U, Shetty VP, Antia NH. Demonstration of mycobacterial antigens in nerves in tuberculoid leprosy. *Acta Neuropathol*, 1987; **73:** 387–392.

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Modified Active Surveillance System (MASS); a novel clinicopathological evaluation of PB leprosy patients after RFT, in Mangalore, India

R. MANJUNATH, K. NARENDRA KAMATH, GANESH S. PAI, JEROME PINTO & VIMALA VINOD
Department of Skin & Sexually Transmitted Disease, Kasturba Medical College, Mangalore 575 001, India

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Summary The current recommendations for leprosy control programmes include stopping active surveillance in view of the very low relapse rates and a phased integration of leprosy services with the general health services. Passive surveillance may not be adequate, more so because of the introduction of newer, shorter drug regimens. This study is an effort to evolve a modified active surveillance, which is cost-effective, simple and also a novel substitute for the increased workload caused by the dwindling number of PMWS. One thousand one hundred RFT-PB leprosy patients were recalled for a review under the Modified Active Surveillance System (MASS), carried out over two phases. Patients were divided into groups as per the mode of response to the mailed postcards; Responders (patients who reported to the OPD in person), Untraceables (patients whose postcards returned back) and non-responders (patients who did not report to the OPD after receiving the mail). At the end of phase I, we had 120 Responders, 480 Untraceables and 500 Non-responders. In phase II, which began 2 months later, the 500 non-responders were dispatched reminders. In this phase, there were 31 responders, 60 untraceables and 409 non-responders. Thus, at the completion of phases I and II, there were 151 responders, 540 untraceables and 409 non-responders. Of the 151 patients examined, 71 had no complaints (category 1), 41 had fresh leprosy-related complaints (category IIA), 14 had fresh leprosy-unrelated complaints (category IIB) and 25 had persistence of old complaints (category III). Cumulative PYR of the 151 patients was 1155·42. Forty-one patients had fresh leprosy-related complaints. Skin biopsy was done in the 17 patients with fresh skin patches, of whom four showed histopathological evidence of relapse. Relapse rate in our study was 0·35/100 PYR. Mean duration after RFT at relapse was 4·9 years. Our scepticism towards passive surveillance systems is justified by these 41 patients with fresh leprosy-related complaints, who voluntarily reported only after receiving the postcards. We recommend the introduction of a phase III, wherein the services of PMWs may be used to contact the 409 patients who remained unresponsive at the completion of phases I and II. We also recommend the introduction of a universal format for recording addresses of all new patients,

Correspondence to: R. Manjunath, c/o Dr Ganesh S. Pai, DERMA-CARE, Trade Centre, Bunt's Hostel Road, Mangalore 575 001, India (e-mail: gspai@satyam.net.in)

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which would be of immense help in patient retrieval in all such surveillance systems in the future.

Introduction

Decline in the statistical parameters of leprosy due to the highly efficacious WHO-MDT has altered the disease scenario at the community level. Two of the current recommendations are bound to create significant changes in the functional modalities of leprosy programmes. They are:

1. Recommendation to stop active surveillance because of the very low risk of relapse.[1]
2. Integration of leprosy activities with the general health services because of the low prevalence at the community level.[2,3]

A passive surveillance system[4] is not entirely justifiable, especially in view of the newer, shorter, drug regimens.[5] Therefore, the current need is for modified active surveillance, which would be comprehensive, cost-effective and simple.

Our study focused on the evaluation of all RFT-PB cases spread over six urban leprosy centres (ULC) in the city of Mangalore. As a modification, the postman was a substitute for the paramedical worker (PMW), while patients were recalled to the OPD for review by mailing cost-effective postcards.

Dwindling fund resources and a likely reduction of field force are likely to disturb the existing surveillance systems. The postcard system should therefore be a good alternative.

Materials and methods

Our study aimed at evaluating PB leprosy patients after RFT, with an emphasis on detection of relapses.

The addresses of PB leprosy patients released from therapy in the last 20 years were collected from the RFT registers of the six ULCs of Mangalore City. Patients who had not completed 6 months after RFT were excluded.

One thousand one hundred RFT-PB leprosy patients were recalled for a review under the Modified Active Surveillance System (MASS), carried out over two phases. In the first phase, 120 patients had responded and 480 postcards were returned back, from which it was discovered that 290 patients had either moved away or were deceased and 190 patients had an inadequate postal addresses. Five hundred patients did not respond.

In the second phase, which began 2 months later, the 500 non-responders were sent reminders. Interestingly, 31 patients responded in person, 60 postcards were returned back citing the same reasons as in phase I and 409 patients still remained unresponsive.

In both phases, for the patients who responded, a comprehensive leprosy-related clinical evaluation was done. Relevant investigations, including skin biopsy, were performed when indicated. Of the 151 responders, 41 had fresh leprosy-related complaints, mainly nerve thickening and neurological deficit, and this group was evaluated by a neurologist. Nerve conduction studies were done in necessary cases.

The responders were placed in age cohorts of 10 years each. Five-year cohorts were done with respect to years since RFT, for a time trend analysis.

Criteria for relapse in our study was clinical (appearance of new lesions, renewed activity of the old lesions or evidence of new nerve function loss) with histological evidence of leprosy. Type 1 late reversal reaction was ruled out clinically and after a therapeutic challenge with oral corticosteroids administered over 2–4 weeks.[6]

The statistical guidelines used were according to person years at risk (PYR). Relapse rate is calculated using the formula:[7]

$$\text{Relapse rate/100 PYR} = \frac{\text{Number of relapses}}{\text{Cumulative PYR of all the patients evaluated}} \times 100$$

Observations and results

According to the mode of response to the mailed postcards, the patients were divided into three groups (Table 1); Responders, Untraceables and Non-responders. Responders were patients who reported in person for evaluation on receipt of the postcard. Untraceables were those where the mails returned back by the postal department. The reasons attributed to the return were that the patients were either deceased or had moved away or had a grossly inadequate address. Non-responders included those who received the mail but failed to respond.

The 151 patients who responded were divided into three categories (Table 2). Category I included those patients with no complaints. Category II included patients who had complete resolution of their leprosy lesions after therapy but subsequently developed fresh lesions. Patients with fresh leprosy-related complaints were included in category IIA. Some of these fresh lesions were unrelated to leprosy such as eczemas, vitiligo, chronic urticaria, *T. versicolor* and dermatophytoses. These dermatoses were segregated as category IIB. Category III patients included those who never had a complete resolution of their leprosy lesions, at any time after RFT.

Cohort analysis of the 151 cases studied with respect to the duration since RFT was: 28 cases (18·48%) in the last 5 years (between 1995 and 2000), 64 cases (42·24%) between the last 5 and 10 years (1990 to 1995), 53 cases (34·98%) between the last 10 and 15 years

Table 1. Observed mode of response

	Phase I	Phase II	Total
Total number of patients to whom postcards were mailed	1100	500**	1100
Responders (patients who reported to the OPD in person)	120	31	151
Untraceables (patients whose postcards returned back)	480	60	540
• Patient deceased/moved away	290	60	350
• Inadequate postal addresses	190	–	190
Non-responders (patients who did not report to the OPD after receiving the postcard)	500	409	409

**These 500 are the same non-responders at the end of phase I.

Table 2. Categories of patients who responded

	Phase I	Phase II	Total
Category I Patients with no complaints	55 (440·24)*	16 (128·01)	71 (568·25)
Category II Patients with fresh complaints	43 (372·06)	12 (84·96)	55 (457·02)
IIA Related to the disease	34 (300·80)	7 (51·13)	41 (351·93)
II B Unrelated to the disease	9 (71·26)	5 (33·83)	14 (105·09)
Category III Patients with persistent old complaints	22 (121·16)	3 (8·99)	25 (130·15)
Grand total	120 (933·46)	31 (221·96)	151 (1155·42)

*Figures in brackets are the cumulative PYR** of each category.
**PYR = Person Years at Risk = Date of relapse/clinical evaluation—Date of RFT
Cumulative PYR of a group = Sum of all the individual PYR.

(1985 to 1990) and six cases (3·96%) between the last 15 and 20 years (1980 to 1985) (Figure 1).

By age, the cases were classified as 10-year cohorts. There were 40 cases (31·35%) in the 7–20 years age group, 39 cases (25·74%) in the 21–30 years age group, 21 cases (13·86%) in the 31–40 years age group, 15 cases (9·9%) in the 51–60 years age group, nine cases (5·94%) between 61 and 70 years and one patient (0·66%) aged 85 years (Figure 2).

Forty-one patients had fresh leprosy-related complaints [category IIA (Table 3)]. Of these, 17 had new skin lesions, of which 11 were associated with nerve thickening. Histopathological evidence of leprosy in the form of granulomas composed of epitheloid cells, lymphocytes and Langhan's giant cells infiltrating dermal nerves and other adnexa were detected in four cases.

Past recording of neurological involvement was inadequate, as it was often carried out by PMWs in field conditions. Current evaluation revealed tingling/numbness (24 patients), footdrop (three patients), weakness (eight patients), nerve thickening (11 patients) and trophic ulcers (three patients) (Table 3). These patients belonged to category IIA. This

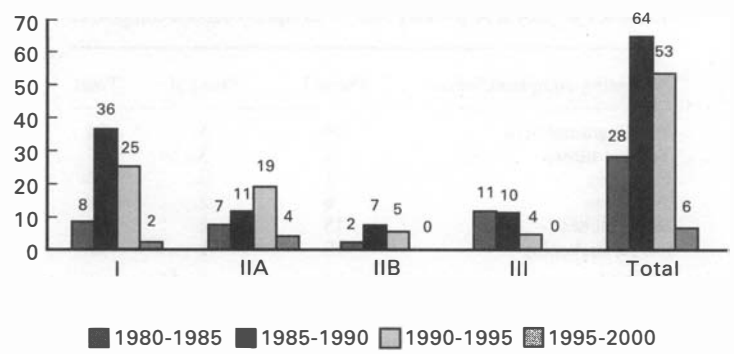

Figure 1. Cohort analysis of cases studied with respect to duration since RFT.

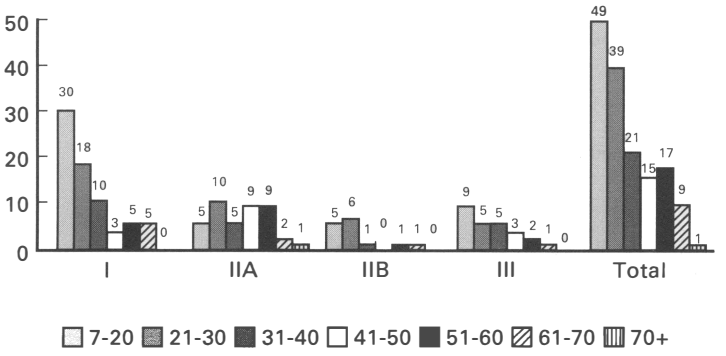

Figure 2. Classification of cases by age.

group of 41 patients with fresh leprosy-related complaints was evaluated by a neurologist and nerve conduction studies were also done. Significant reduction in velocity of propagation was observed in all the cases. Of these, only four cases showed histopathological evidence on multiple biopsies.

These four patients were labelled as biopsy proven relapses (Table 4) as per our criteria. The other 13 patients were labelled as suspected relapses, and are under observation.

Rate of relapse in our study was 0·35/100 PYR.

Mean duration at relapse was 4·9 years after RFT.

Discussion

In 1998, more than 500,000 new cases were reported in India, which accounted for 69% of the global incidence.[8] A problem of this magnitude certainly calls for effective resource utilization towards newer case detection.

The magnitude of community health problems posed by undetected relapses far exceeds the picture reflected by the low number of detected relapses. In our study, we have 41 patients with fresh onset leprosy-related complaints after RFT (Table 3). None of these patients had

Table 3. Category II A: patients with fresh leprosy-related complaints

Presenting complaint/lesion	Phase I	Phase II	Total
Tingling/numbness	19	5	24
Trophic ulcers	4	3	7
Foot drop	1	2	3
Weakness	6	2	8
Skin patches	15	2	17
Nerve thickening	10	1	11
Total*	34	7	41

*Figures shown in 'Total' indicate the total number of patients and not the sum of all the individual complaints, due to the presence of multiple complaints in some patients.

Table 4. Biopsy proven relapses

Patient Age(yrs)/sex	Duration after RFT at relapse	Past therapy	Type of relapse
47/M	1 year 4 months	MDT	TT
38/M	5 year 7 months	MDT	BT
60/M	11 year 10 months	MDT	BT
24/F	1 year 1 month	MDT	BT

voluntarily reported before getting a reminder in the form of a postcard. This justifies our scepticism towards passive surveillance systems relying upon patient awareness and self-reporting.

The large number of non-responders (409 patients) is the major drawback in this form of MASS. This is probably because the patients are daily wage earners and would not visit the hospital as they have no fresh complaints. Reinforcing their attitude is the counselling of patients at the time of RFT, when they are told that they are free from the disease.

Hence we propose the introduction of a phase III, wherein the services of paramedical workers are to be used to trace the non-responders. The onus of the total dependability on PMWs for surveillance needs to be reviewed in the future scenario of a gradual phasing out of leprosy programmes. Our system should reduce the workload for an already overburdened workforce. The reduction in surveillance costs achieved by our study had become necessary as most of the NGO funds are being diverted to programmes on HIV and malaria.

We also recommend the introduction of a universal format for recording the present and permanent addresses of all new patients. This step will further increase the patient response in such modified active surveillance methods in future.

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References

[1] WHO. *Risk of relapse in leprosy*. Leprosy Unit, Division of Control of Tropical Diseases, WHO, Geneva, Switzerland. WHOICTD/LEP/94.1.

[2] Feenstra P. Leprosy control through general health services and/or combined programmes. *Lepr Rev*, 1993; **64:** 89–96.

[3] Saunderson PR. Joint tuberculosis/leprosy programmes. *Lepr Rev*, 1997; **68:** 2–9.

[4] Daumeric D, Panniker V. Issues in evaluating information on relapse in leprosy (symposium paper). *Ind J Lepr*, 1995; **67:** 27–33.

[5] Ministry of Health and Family Welfare, Directorate General of Health Services (Leprosy Division), Government of India. *Modified guidelines on MDT regimen to be followed under NLEP*. No. L 19025/6/97-Lep; Nirman Bhavan, New Delhi; 1997: 1–10.

[6] Boerrigter G, Ponnighaus JM, Fine PEM, Wilson RJ. Four year followup results of a WHO-recommended multiple drug regimen in paucibacillary leprosy patients in Malawi. *Int J Lepr*, 1991; **59:** 255–261.
[7] Trever CS, Jan HR. Relapse rates in patients treated with dapsone monotherapy and combinations of dapsone and thiambutosine, thiacetazone, isoniazid and streptomycin in the pre-MDT era. *Int J Lepr*, 1994; **62:** 353–358.
[8] Gillis WL. Editorial. *Int J Lepr*, 1999; **67:** 60–63.

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Increase in the incidence of dapsone hypersensitivity syndrome – an appraisal

P. NARASIMHA RAO & T. S. S. LAKSHMI
Department of Dermatology, Osmania Medical College/Hospital Hyderabad. India

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Summary There has been an increase in the reports of dapsone hypersensitivity syndrome (DHS) in the past few years, coinciding with the introduction of multidrug therapy (MDT) for leprosy world-wide. The exact cause of this phenomenon is not clear. We report four cases of DHS observed among 252 leprosy patients on MDT and one case of DHS in a patient taking dapsone for nodulocystic acne in the Dermatology Department of the Osmania General Hospital, Hyderabad, India, between June 1997 and January 1999 with few unusual features. In two of these five patients maculopapular rash was severe and progressed to erythroderma.

Introduction of MDT in 1982 has not only decreased the prevalence of leprosy but also brought about a positive change in the attitude of people which increased the voluntary reporting of leprosy patients. This, coupled with improvements in organization of leprosy control and awareness among medical personnel of DHS, are probably the most important reasons for the increased reporting of DHS in recent years.

Introduction

The terms hypersensitivity and drug allergy describe the allergic reaction that results from sensitization to a particular chemical or a structurally similar one.[1] Dapsone hypersensitivity syndrome (DHS) is such a reaction, with the median latency, i.e. the time interval from the commencement of therapy to manifestation of the hypersensitivity reaction, of about 4–7 weeks. The manifestations of DHS include some or all of the following: fever, eosinophilia, mononucleosis, lymphadenopathy, hepatitis and exanthematous skin eruption which may progress to a dermatological emergency and rarely to death.[2,10]

In the early years after introduction of dapsone to clinical use, this reaction was reported frequently. However, hypersensitivity reactions to dapsone virtually disappeared from the literature between 1956 and 1980.[3] After 1980, the number of case reports of DHS increased, coinciding with the introduction of multidrug therapy (MDT) for leprosy. Dapsone, apart from being a first line drug in the treatment of leprosy, is also used in the treatment of many

Correspondence to: P. Narasimha Rao, B-48, Income Tax Colony, Hyderabad, India 500 028

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other dermatological disorders such as vesiculobullous dermatoses, cutaneous vasculitis, polyarteritis nodosa, nodulocystic acne and cutaneous mycetoma. In all these conditions, dapsone is used at doses of 100 mg/day or more and DHS has also been reported in these patients.[3,10]

Here we report five cases of DHS with few unusual features which were observed in our department during a period of 18 months, and discuss the possible causes of increase in its incidence.

In the Dermatology Outpatient Department of the Osmania General Hospital, Hyderabad, India, a total of 252 leprosy patients were enrolled between June 1997 and January 1999 (165 males and 87 females). All patients were put on WHO recommended MDT. Among these, four patients developed DHS necessitating hospitalization and discontinuation of dapsone. DHS also occurred during the same period in a patient with nodulocystic acne who was treated with dapsone 100 mg/day. None of the patients who were diagnosed as DHS had any other systemic illness. All the leprosy patients who developed DHS were only on MDT and the only systemic medication that the patient with nodulocystic acne was taking was dapsone.

Observations

In all five patients (Table 1), DHS was observed during the first 5 weeks of treatment and the dose of dapsone was 100 mg/day. Two of the leprosy patients were on multibacillary MDT, whereas the other two were on paucibacillary MDT. The average duration of dapsone therapy before the appearance of DHS was 16 days. Features of DHS were observed earliest in patient 5, within 2 days, who gave a past history of skin rashes with syncope after taking PB MDT for 2 months at another leprosy clinic before the therapy was discontinued.

All five patients had fever, maculopapular rash associated with itching and generalized lymphadenopathy. Jaundice and hepatomegaly were not present in patient 5, who had taken dapsone only for 2 days, whereas these symptoms were observed in all the other four patients who had taken dapsone for more than 2 weeks. In two of the five patients, maculopapular rash was so extensive and severe as to have progressed to a state of 'erythroderma'. In patient 3, tender hepatomegaly was associated with deep jaundice and pulmonary involvement. The signs and symptoms of DHS were mildest in case 5, who had only been treated with dapsone for 2 days.

Liver function tests revealed increased serum levels of bilirubin and SGPT in all four patients with florid DHS. Reversal of serum albumin/globulin ratio was also observed in all four patients. Serological tests for hepatitis B and C were negative and glucose-6-phosphate dehydrogenase levels were within normal limits in all five patients.

Biopsies of the inflamed skin taken from the two patients who had progressed to erythroderma revealed a predominantly mononuclear cell collection around adnexa and small blood vessels. None of these patients demonstrated either leucocytosis or eosinophilia.

Treatment

Patients with DHS were hospitalized and all the drugs withdrawn, including dapsone. Patients 1 and 3 were put on systemic corticosteroids (30 mg prednesolone per day). Patients 2, 4 and

Table 1. Clinical features

Clinical features	Patient no.				
	1	2	3	4	5
Age and sex	50 years F	18 years M	22 years M	25 years M	35 years F
Diagnosis	LL leprosy	BT leprosy	Indeterminate leprosy	Nodulocystic acne	BT leprosy
Therapy	MB MDT	PB MDT	PB MDT	Dapsone + topical therapy	MB MDT
Duration of therapy before appearance of DHS (days)	20	33	15	12	2
Fever	+	+	+	+	+
Jaundice	+	+	+	+	–
Itching	+	+	+	+	+
Lymphadenopathy	+	+	+	+	+
Maculopapular rash	+	+	+	+	+
Hepatomegaly	+	+	+	+	+
Splenomegaly	–	–	–	+	–
Erythroderma	+	+	–	–	–
Respiratory symptoms	–	–	Crepitations +	–	–

5 were put on antihistamines, as well as supportive therapy. All the patients recovered completely. In the patients on corticosteroids, cutaneous lesions resolved in 1–2 weeks (average 9 days), whereas in patients on antihistamines, skin lesions subsided in 2–3 weeks (average 16 days). Corticosteroids were tapered over period of 4 weeks.

Biochemical parameters returned to normal in all patients within 6–8 weeks. None of the patients was rechallenged with dapsone. Clofazamine was substituted in the place of dapsone in patients on paucibacillary MDT. In patients on multibacillary MDT, dapsone was stopped and rifampicin and clofazamine were continued.

Discussion

Due to the increase in the number of case reports, there has been revival of interest in DHS recently. Moreover, dapsone as a drug has become more important world-wide after recognition of its value in the chemoprophylaxis and treatment of toxoplasmosis and *Pneumocystis carini* pneumonia in HW infected patients.[4]

DHS is considered as a diagnosis when sudden onset of generalized maculopapular skin rash associated with systemic symptoms, jaundice, haematological abnormalities and generalized lymphadenopathy occurs in patients on dapsone during the first few weeks of its intake. The maculopapular rash with its underlying erythema in severe cases can be associated with inflammation, swelling and later desquamation of the skin. DHS is a clinical diagnosis, as there is no confirmatory laboratory test available currently. No other drug used in MDT of leprosy is known commonly to produce such a reaction.

Although classic DHS can present with all the clinical features, incomplete and atypical forms are encountered occasionally.[13] The commonly associated clinical features of DHS which are exantheni, fever lymphadenopathy and signs of hepatic dysfunction, were also observed in this study.[6,10] However, haematological changes such as leucocytosis, eosinophilia and atypical lymphocytosis often associated with DHS[11] were not observed in the present study.

Erythroderma is the term applied to any inflammatory skin disease that affects more than 90% of the body surface area. Erythroderma is observed in severe DHS, the incidence ranging from 76.5% to 22.2% as reported in various studies.[6,11] No correlation between the dosage of dapsone and the occurrence and severity of DHS was observed in various studies.[3,7] In the present study, two out of five patients with DHS progressed to erythroderma, after taking dapsone for longer periods before the appearance of DHS (33 and 20 days) compared to the others in the study group. It is also interesting to note that DHS was mildest in patient 5, who had discontinued dapsone on the second day of starting therapy due to prior experience of dapsone sensitivity. It was observed that accidental challenge with dapsone often causes mild symptoms of DHS in patients with hypersensitivity to dapsone, which confirms the diagnosis of this syndrome.[6] These observations indicate that although the severity of DHS is not dose related, it may be affected by the duration of administration of dapsone after sensitization.

DHS is usually confined to the first 6 weeks of treatment with dapsone. However, it can appear as early as 6 h in previously sensitized individual to as late as after 6 months of therapy.[11] In the present, study patient 5, who had past history of dapsone hypersensitivity, developed DHS within 2 days of starting dapsone. It is important to distinguish true early

onset DHS from phototoxic and photoallergic reactions to dapsone, which can also occur during the first 24–48 h of taking the drug in sensitized individuals.[12]

The exact incidence of DHS is not known. Lowe, who was first to report DHS in leprosy patients in 1950 from Nigeria, noted the frequency of dapsone syndrome as 2%.[5] An incidence of 1.3% was reported in a study from India.[6] In the present study, the incidence of DHS among leprosy patients is 1.6%. Richardus and Smith[7] reported an incidence of DHS of 0.3–0.6% during monotherapy and 3.6% after introduction of MDT. Such an increase in the incidence of DHS after introduction of MDT was also reported by other workers. DHS was observed in three out of 24 new cases (12.5%) who had taken MDT between 1986 to 1995 in a province of China, whereas it was encountered in none of the 1357 leprosy patients before introduction of MDT.[8] Smith[3] observed a sudden reappearance and increase of reports of DHS between 1980 and 1986 (27 cases), coinciding with the introduction of MDT for leprosy after the relative absence of cases between 1956 and 1980. However, he also noted that in 16 out of 27 cases, patients were on dapsone monotherapy.

Although unequivocal proof is lacking at present, the interaction of dapsone with other MDT drugs, especially rifampicin, as a cause of DHS cannot be ruled out completely. A post mortem histological examination of a DHS patient demonstrated features consistent with drug induced hepatitis, tubulo-interstitial nephritis and myocarditis.[9] Although these could have been caused by dapsone toxicity, it was thought that a concomitant adverse reaction to refampicin, which is known to be hepatotoxic and nephrotoxic and possibly capable of predisposing to dapsone syndrome, could not be excluded.

In a review of all published cases of dapsone hypersensitivity world-wide from 1949 to 1995, the authors found only 103 reported cases of DHS during this period of 46 years.[10] Of these, the highest numbers of cases of DHS were reported after 1980, coinciding with the introduction of MDT for leprosy.[3,6–8] From the Medline scan and *Indian Journal of Leprosy* we picked out 24 cases of DHS from seven reports published between years 1996 and 1998, in contrast to the total number of 103 patients of DHS reported in world literature during the period 46 years between 1949 and 1995.

This apparent recent increase in DHS case reports depends on multiple factors, some of may include the following:

1. Increased patient compliance and clinic attendance after introduction of MDT[14] which brought about better detection of DHS than before.
2. The introduction of MDT has also contributed to the improvements in the organization of leprosy control programmes[15] and level of job satisfaction in the health personnel,[16] which could have resulted in the increased reporting of DHS.
3. Awareness of DHS among medical personnel due to increased usage of dapsone in various indications other than leprosy.

It may be of interest to note that all these recent cases of DHS in leprosy patients between 1996 and 1998 were reported from departments of Dermatology, Medicine, Pharmacology or Forensic Medicine of teaching hospitals or institutions and none from leprosy clinics. This probably reflects the increased utilization of services of tertiary medical care centres by leprosy patients, resulting from the increased referral of these patients for specialist care. The recent increase in the reporting of DHS from these centres indicates the increased interest of allied medical specialties in DHS, apart from the fact that these centres are better equipped than a peripheral leprosy clinic to investigate, record and report such cases systematically.

References

[1] Klaassen CD. Principles of toxicology. In: Gilman AG, Rall TW, Nies AS *et al.* (eds) *The pharmacological basis of therapeutics*, 8th edition. McGraw Hill, NY, 1992, pp 49–61.
[2] Jopling WH. Side effects of anti-leprosy drugs in common use. *Lepr Rev*, 1983; **54**: 261–270.
[3] Srnith WCS. Are hypersensitivity reactions to dapsone becoming more frequent? *Lepr Rev*, 1988; **59**: 53–58.
[4] Gold JWM. The diagnosis and management of HIY infection. *Med Clin N Am*, 1996; **80**: 1283–1307
[5] Lowe J. Diaminodiphenyl sulphone in the treatment of leprosy. *Lancet*, 1951; **1**: 1343–1344.
[6] Rege VL Shukia P Mascarenhas W. Dapsone syndrome in Goa. *Ind J Lepr*, 1994; **66**: 59–64.
[7] Richardus JJ Smith TC. Increased incidence in leprosy of hypersensitivity reactions to dapsone after introduction of multidrug therapy. *Lepr Rev*, 1989; **60**: 267–273.
[8] Guanwei G. DDS syndrome when taking NMT. *China Lepr J*, 1995; **11**: 200–201.
[9] Lau G. A fatal case of drug induced multi-organ damage in a patient with Hansen's disease. Dapsone syndrome or rifampicin toxicity? *Forens Sci Int*, 1995; **73**: 109–115.
[10] Wogel G, Gobel K, Blasum C. Hypersensitivity syndrome to dapsone – an epidemiological review. In: *Abstracts of ILEP 15th International Leprosy Congress*, Beijing, 1998, CH-31.
[11] Kumu RH, Kumar MV, Thapa DM. Dapsone syndrome – a five year retrospective analysis. *Ind J Lepr*, 1998; **70**: 271–276.
[12] Joseph MS. Photodermatitis provoked by dapsone. A case report. *Lepr Rev*, 1987; **58**: 425–428.
[13] Jaswal R, Thami GP, Kanwar AJ. Dapsone syndrome – an incomplete form. *Ind J Lepr*, 1998; **70**: 229–230.
[14] Gupte MD. Elimination of leprosy: forecasts and projections. *Ind J Lepr*, 1994; **66**: 19-35.
[15] Noordeen SK. Elimination of leprosy as a public health problem. *Ind J Lepr*, 1994; **66**: 1–10.
[16] Premkumar R, Dave SL. Impact of multidrug therapy on health personnel in their level of job satisfaction. *Ind J Lepr*, 1993; **65**: 429–438.

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Effect of unique *Mycobacterium leprae* phenolic glycolipid-I (PGL-I) on tumour necrosis factor production by human mononuclear cells

R. CHARLAB**, E.N. SARNO*, D. CHATTERJEE*** & M.C.V. PESSOLANI*

**Leprosy Laboratory, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil*

***Centro Brasileiro de Pesquisas Físicas/CNPq, Rio de Janeiro, Brazil*

****Department of Microbiology, Colorado State University, CO, USA*

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Summary *Mycobacterium leprae* cell wall-associated components are found in large amounts in the tissues of leprosy patients, particularly those at the lepromatous pole. Among these molecules, the phenolic glycolipid-I (PGL-I), unique to *M. leprae*, has been involved in the selective anergy observed in the lepromatous patients. Armadillo-derived *M. leprae* retains only a small proportion of the total PGL-I found in infected tissues. Therefore, the addition of PGL-I to *M. leprae in vitro* is important for a better understanding of *M. leprae* effects *in vivo.* We have studied the influence of PGL-I on TNF production by normal human peripheral blood mononuclear cells (PBMC) and by a human monocytic leukaemia cell line (THP-1) following stimulation with killed *M. leprae*. PGL-I alone did not induce TNF secretion by PBMC, but when associated with a sub-optimal dose of armadillo-derived *M. leprae* increased the release of this cytokine. In agreement with these results, *M. leprae*-exposed THP-1 cells did not secrete detectable levels of TNF unless PGL-I was simultaneously added to the culture. This increase in TNF production suggests that PGL-I plays a role in the induction of TNF during the natural infection. In addition, the modulatory effect of PGL-I on TNF release by THP-1 cells reinforces that monocytes are one of the possible targets of this molecule.

Introduction

Glycolipids from the cell wall of *Mycobacterium leprae* are widely distributed in the tissues of patients with leprosy.[1,2] Among these components, the phenolic glycolipid 1 (PGL-I),

Correspondence to: M.C.V. Pessolani, Leprosy Laboratory, Oswaldo Cruz Foundation. Av. Brasil, 4365, Manguinhos, CEP 21045-000 Rio de Janeiro, Brazil (Tel: +55-21-2709997, Fax: +55-21-2709997, e-mail: cferrer@gene.dbbm.fiocruz.br)

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unique to *M. leprae*,[3,4] has been implicated in the selective anergy observed in lepromatous leprosy patients.[5] *In vitro* findings indicate that PGL-I interferes with the cellular immune response at the level of both macrophage function (measured by the release of inflammatory cytokines such as TNF and by generation of oxygen radicals) and lymphocyte proliferation.[5–9] Moreover, PGL-I fixes selectively the complement component C3 and thus helps to mediate *M. leprae* phagocytosis via complement receptors on the surface of mononuclear phagocytes.[10,11]

In mycobacterial infections TNF is involved in both host protective response and disease pathology.[12] Whole mycobacteria and/or cell wall associated components stimulate TNF release by murine macrophages and by mononuclear cells from normal individuals or leprosy patients *in vitro*.[13–16] However, little is known of the factors which trigger TNF release during the course of the disease. We have studied the influence of PGL-I on TNF production by normal human peripheral blood mononuclear cells (PBMC) following stimulation with *M. leprae*. Alternatively, THP-1 cells, a human myelomonocytic cell line that lately has been extensively employed in studies of mechanisms of maturation/differentiation from monocytes to macrophages,[17] were used as host cells.

M. leprae PGL-I is an extracellular product forming a loose capsule around the bacillus which is partially lost during the purification procedure from infected tissues.[4] It therefore seems necessary to add PGL-I to the armadillo-derived *M. leprae* for a better understanding of *M. leprae* effects *in vivo*. In addition, this approach helps to clarify PGL-I contribution to these effects. Our results suggest that PGL-I acts as a co-signal for TNF production by *M. leprae*-exposed mononuclear cells, but has no detectable effect when added alone to unstimulated cultures. The possible relevance of these *in vitro* results to the leprosy pathogenesis is discussed.

Materials and methods

SOURCE OF REAGENTS

RPMI 1640 medium, penicillin/streptomycin, L-glutamine were obtained from Gibco BRL (Gaithersburg, MD, USA), heat-inactivated fetal calf serum was purchased from Fazenda Pigue (Rio de Janeiro, RJ, Brazil), Ficoll was obtained from Sigma Chemical Co. (St Louis, MO, USA).

PBMC CULTURE AND STIMULATION

Peripheral blood mononuclear cells (PBMC) were isolated from six healthy individuals, five of them living in Rio de Janeiro and one being a visitor from the UK. PBMC were separated by Ficoll-Hypaque density gradient from sterile heparinized blood, washed three times in PBS and resuspended in RPMI-1640 medium supplemented with 5% AB human serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 10 mM Hepes and 2 mM L-glutamine. Two culture conditions were used, as follows: (i) 3×10^5 cells in 0·2 ml medium/well were dispensed in 96-well flat-bottomed microtitre plates or (ii) 2×10^6 cells in 1 ml medium/well were dispensed in 24-well microtitre plates and cultured with or without stimulants (as below) for 24 h at 37°C in a humidified atmosphere of 5% CO_2.

THP-1 CULTURE AND STIMULATION

Cells from a human myelomonocytic cell line, THP-1 (American Type Culture Collection, Rockville, MD, USA), were maintained in suspension cultures in RPMI medium supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 10 mM Hepes and 2 mM glutamine, at 37°C in a humidified atmosphere of 5% CO_2 and used between three and 14 passages. For the experiments, 2×10^5 cells in 1 ml of the same medium/well were dispensed in 24-well microtitre plates and cultured with or without stimulants up to 48 h at 37°C in a humidified atmosphere of 5% CO_2. After the incubation period, the culture supernatants of PBMC or THP-1 cells were collected for TNF quantitation.

STIMULANTS

Irradiated armadillo-derived *M. leprae* and PGL-I were obtained as described.[4] Evaluation of PGL-I for the presence of Gram-negative bacterial endotoxin (LPS) was done with the amebocyte assay (QCL-1000 kit, BioWhittaker, Inc., Walkersville, MD, USA). Two lots of PGL-I obtained between 1994 and 1997 contained <0.5 pg of LPS per μg of PGL-I per batch. Because of its insolubility in aqueous medium, PGL-I was presented to PBMC or THP-1 cells immobilised onto the microplate well. Briefly, PGL-I stock solutions in ethanol were added to culture wells and dried under sterile conditions before cell addition. The same concentration of ethanol was added to control wells, although ethanol alone had no detectable effect in our conditions. PGL-I final concentration in cultures was 1 or 25 μg/ml; *M. leprae* was presented to PBMC or THP-1 cells over a concentration range of 1.25–40 μg/ml (approximately 3×10^6–10^8 organisms/ml).

TNF DETECTION

Supernatants were adequately diluted in PBS and assayed in duplicates. TNF levels were determined by ELISA using specific pairs of monoclonal antibodies and human recombinant TNF as standard, according to the manufacturer recommendations (Pharmingen, San Diego, CA, USA). The limit of sensitivity was 250 pg/ml of recombinant TNF.

STATISTICAL ANALYSIS

The Friedman test and Wilcoxon signed ranks test were performed with data shown in Figure 1. Data related to Figure 2 are reported as means ± SEM.

Results

EFFECT OF PGL-I ON TNF PRODUCTION BY PBMC STIMULATED WITH *M. LEPRAE*

We compared TNF levels produced by PBMC simultaneously treated with PGL-I and *M. leprae* to the levels produced by mononuclear cells exposed to *M. leprae* alone (Figure 1). Optimal incubation time for TNF secretion was initially determined by stimulating the cells with *M. leprae* for 4, 12, 24 and 48 h, with a peak level observed at 12–24 h (not shown). PBMC did not release detectable amounts of TNF into cell medium when cultivated for the same periods of time in the presence of PGL-I or medium alone. The stimulation of PBMC cultures with *M. leprae* induced TNF release by cells of all tested subjects as previously

shown by others.[15] However, the profile of TNF release in response to *M. leprae* exposure varied substantially among the different individuals over the concentration range used. In the presence of the *M. leprae* dose that induced the lowest amount of TNF for each subject (sub-optimal *M. leprae* dose), PGL-I enhanced TNF levels in all subjects (Figure 1, $P < 0{\cdot}009$). When other *M. leprae* doses were used, PGL-I had complex effects, either increasing or decreasing the TNF release in the same individual (not shown). Both PGL-I doses (1 and 25 μg/ml) induced comparable levels of TNF when added to *M. leprae*-stimulated PBMC ($P < 0{\cdot}65$). Subjects a, b, c, d and f were previously exposed to *M. leprae*, differently from subject e, who lives in a non-endemic area. Subject e-derived PBMC, compared to the other subjects, produced lower levels of TNFα in response to all tested doses of *M. leprae*. Removing the values referring to this subject from the statistical analysis, the difference in TNF production between PBMC challenged with *M. leprae* and PBMC challenged with *M. leprae* and PGL-I is still significant ($P < 0{\cdot}023$).

THP-1 cells are functionally very similar to peripheral blood monocytes.[18,19] We thus tested the effect of added PGL-I in *M. leprae* induced TNF. PGL-I qualitatively modified the TNF response pattern of THP-1 cells to *M. leprae*. Differently from PBMC (Figure 1), THP-1 cells did not secrete measurable levels of TNF when stimulated with *M. leprae* unless PGL-I was added to the culture. TNF secretion was detected after 48 h of simultaneous exposure to PGL-I and *M. leprae* (Figure 2), but not at early time points. THP-1 cells also showed similar response kinetics to PMA, followed by morphological criteria[19] and measurement of TNF levels in culture supernatants (M. M. Oliveira, R. Charlab and M. C. V. Pessolani, submitted). As observed before, PGL-I alone did not stimulate the production of measurable levels of TNF (Figure 2).

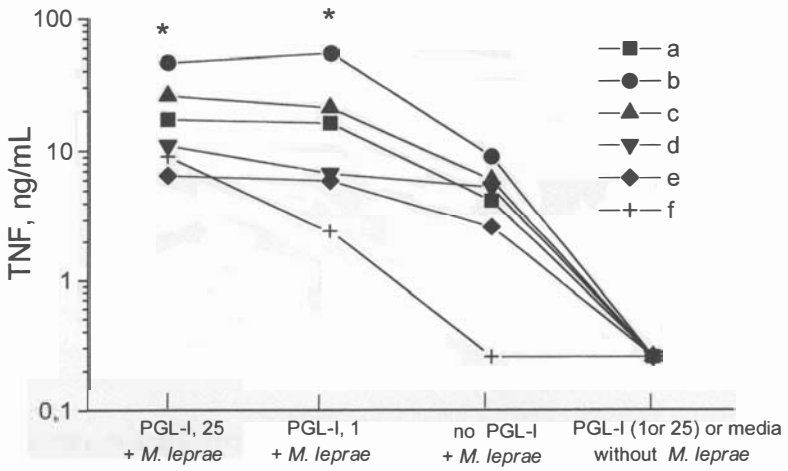

Figure 1. Effect of PGL-I on TNF production by *M. leprae*-stimulated PBMC. PBMC of six healthy subjects (*a*, *b*, *c*, *d*, *e*, *f*; 2×10^6/ml) were incubated with a sub-optimal dose of *M. leprae* for TNF release (over a range of 1.25–40 μg/ml), PGL-I immobilized in the culture microplates (1 and 25 μg/ml) or both for 24 h. TNF concentration in culture supernatants was determined by ELISA. PBMC cultivated without *M. leprae* in the presence of PGL-I or medium alone secreted undetectable levels of TNF. *M. leprae* doses which induced the lowest amount of TNFα for each subject were, respectively: *a* 20 μg/ml; *b* 2 μg/ml; *c* 20 μg/ml; *d* 5 μg/ml; *e* 10 μg/ml; *f* 40 μg/ml. Cultures exposed to PGL-I and *M. leprae* were statistically different from cultures exposed to *M. leprae* alone ($P < 0{\cdot}009$). TNF values of cultures exposed to *M. leprae* and 1 μg/ml of PGL-I were not statistically different from the values of cultures exposed to *M. leprae* and 25 μg/ml of PGL-I ($P < 0{\cdot}65$).

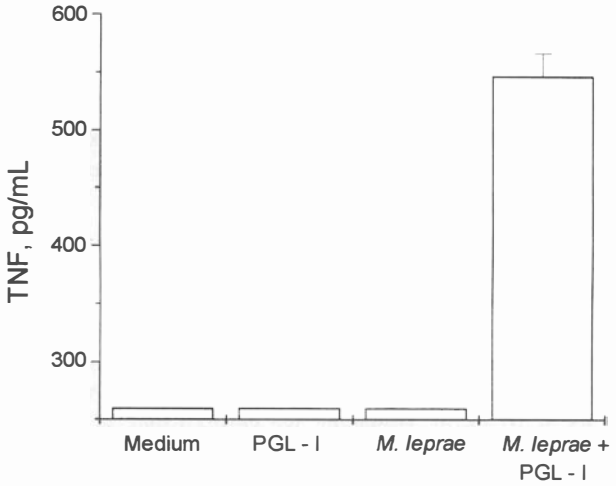

Figure 2. Effect of PGL-I on TNF production by *M. leprae*-stimulated THP-1 monocytic cells. THP-1 cells (2×10^5/ml) were incubated with *M. leprae* (40 μg/ml), PGL-I immobilized in the culture microplates (1 μg/ml) or both for 48 h. TNF concentration in culture supernatants was determined by ELISA. Results are expressed as means ± SE of three independent experiments.

Discussion

It is now well accepted that PGL-I is produced in large amounts during *M. leprae* infection, being found in serum, urine and in the skin lesions of leprosy patients.[1,2,20] This feature allied to its capacity to affect both lymphocyte and macrophage functions (reviewed by Brennan *et al.*[3]) and to elicit a strong antibody response in leprosy patients,[21] points to an involvement of PGL-I in the pathogenesis of *M. leprae*.

To examine whether the addition of PGL-I affects *M. leprae* action *in vitro*, we evaluated TNF production by PBMC of normal individuals or by THP-1 monocytic cells exposed to *M. leprae* in the presence or absence of free PGL-I. In this study, PGL-I alone did not induce TNF secretion by PBMC of healthy individuals or by THP-1 cells, but when associated with *M. leprae* modulated the release of this cytokine. PGL-I increased TNF production by PBMC stimulated with sub-optimal doses of *M. leprae*. However, positive or negative modulatory effects of PGL-I were observed in the different individuals and in the same individual when other concentrations of *M. leprae* were used. These variations may be related to differences in the genetic background (e.g. high or low type TNF producers), functional status of the PBMC donors, heterogeneity of the PBMC population and of the monocyte subset by itself. It is also worthy to mention at this point that we have not adjusted the number of PBMC to turn equal the counts of monocytes to all individuals tested. This should certainly be done if we were working with purified monocytes and not with such a heterogeneous population of cells as PBMC, in which other cell types such as NK and T-cells could also contribute to TNF secretion. Instead, we preferred to use a wide range of *M. leprae* doses for each individual, determining a sub-optimal dose for each one and then use this dose to investigate the effect of PGL-I.

The increase in TNF production observed when PGL-I was added to *M. leprae*-stimulated cultures suggests that this component plays a role in TNF induction during the natural

infection. This observation was reinforced when THP-1 cells were co-cultured with PGL-I and *M. leprae*. We have previously shown that *M. bovis* BCG but not *M. leprae* induced TNFα secretion in THP-1 cells, while both mycobacteria stimulated the release of this cytokine by human PBMC (M. M. Oliveira, R. Charlab and M. C. V. Pessolani, submitted). This result emphasises the potential of THP-1 cells as a model to explore the role of PGL-I on TNF secretion. In the present work, the addition of PGL-I was essential for detection of TNF in the culture supernatants. *M. leprae*-exposed THP-1 cells only secreted detectable levels of TNF when PGL-I was simultaneously present in the culture.

The modulatory effect of PGL-I on TNF production by THP-1 monocytic cells suggests that monocytes are one of the possible targets of this molecule. In this respect it has been shown that PGL-I incorporated in liposomes inhibited the secretion of TNF, IL-1 and IL-6 by LPS-stimulated peripheral blood-derived monocytes from healthy individuals,[22] but, in agreement with our results, had no effect when added alone to non-stimulated controls. The authors used LPS at 10 μg/ml. However, we verified that, at lower LPS doses, PGL-I, either incorporated in liposomes or immobilized in culture microplates was also able to increase TNF production by PBMC of normal subjects. It is worth to mention that undifferentiated THP-1 cells did not secrete detectable levels of TNFα in response to LPS from 100 ng to 10 μg/ml, a behaviour already observed by others.[23] The production of TNF by LPS-stimulated THP-1 cells was not detected even when PGL-I was simultaneously added to the cultures (R. Charlab, unpublished results).

In this study, we showed that PGL-I did not induce the secretion of TNF by PBMC of healthy individuals, but positively or negatively modulated the release of this cytokine induced by *M. leprae*. In this context, one can speculate that PGL-I may have different roles on the pathogenicity along the clinical spectrum of leprosy and in reactional and non-reactional stages of the disease. Depending on the infection status, PGL-I may either increase or suppress the inflammatory response in the infected tissue, contributing, respectively, to sustain a low specific response to *M. leprae* or to enhance tissue damage.

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References

[1] Young DB. Detection of mycobacterial lipids in skin biopsies from leprosy patients. *Int J Lepr*, 1981; **49**: 198–204.

[2] Young DB, Harnisch JP, Knight J *et al.* Detection of phenolic glycolipid I in sera from patients with lepromatous leprosy. *J Infect Dis,* 1985; **152**: 1078–1081.

[3] Brennan PJ, Hunter SW, McNeil M *et al.* Reappraisal of the chemistry of mycobacterial cell walls, with a view to understanding the roles of individual entities in disease processes. In: Ayoub EM, Cassel GH, Branche WC, Henry TJ (eds) *Microbial determinants of virulence and host response.* 1990, pp 55–75.

[4] Hunter SW, Brennan PJ. A novel phenolic glycolipid from *Mycobacterium leprae* possibly involved in immunogenicity and pathogenicity. *J Bacteriol*, 1981; **147**: 728–735.

[5] Mehra V, Brennan PJ, Rada E *et al.* Lymphocyte suppression in leprosy by unique *M. leprae* glycolipid. *Nature*, 1984; **308**: 194–196.

[6] Chan J, Fujiwara T, Brennan P *et al.* Microbial glycolipids: possible virulence factors that scavenge oxygen radicals. *Proc Natl Acad Sci USA,* 1989; **86**: 2453–2457.

[7] Fournie JJ, Adams E, Mullins RJ, Basten A. Inhibition of human lymphoproliferative responses by mycobacterial phenolic glycolipids. *Infect Immun,* 1989; **57**: 3653–3659.
[8] Vachula M, Holzer TJ, Andersen BR. Suppression of monocyte oxidative response by phenolic glycolipid I of *Mycobacterium leprae. J Immunol*, 1989; **142**: 1696–1701.
[9] Prasad HK, Mishra RS, Nath I. Phenolic glycolipid-I of *Mycobacterium leprae* induces general suppression of in vitro concanavalin A responses unrelated to leprosy type. *J Exp Med,* 1987; **165**: 239–244.
[10] Schlesinger LS, Horwitz MA. Phenolic glycolipid-I of *Mycobacterium leprae* binds complement component C3 in serum and mediates phagocytosis by human monocytes. *J Exp Med*, 1991; **174**: 1031–1038.
[11] Schlesinger LS, Horwitz MA. Phagocytosis of leprosy bacilli is mediated by complement receptors CR1 and CR3 on human monocytes and complement component C3 in serum. *J Clin Invest*, 1990; **85**: 1304–1314.
[12] Sarno EN, Sampaio EP. The role of inflammatory cytokines in the tissue injury of leprosy. *Int J Lepr*, 1996; **64**: S69–S74.
[13] Barnes PF, Chatterjee D, Brennan PJ *et al*. Tumor necrosis factor production in patients with leprosy. *Infect Immun*, 1992; **60**: 1441–1446.
[14] Bradbury MG, Moreno C. Effect of lipoarabinomannan and mycobacteria on tumour necrosis factor production by different populations of murine macrophages. *Clin Exp Immunol*, 1993; **94**: 57–63.
[15] Santos DO, Suffys PN, Bonifácio K *et al*. *In vitro* tumor necrosis factor production by mononuclear cells from lepromatous leprosy patients with erythema nodosum leprosum. *Clin Immunol Immunopathol*, 1993; **67**: 199–203.
[16] Susuky K, FukutomiY, Matsuoka M *et al*. Differential production of interleukin 1 (IL-1), IL-6, tumor necrosis factor, and IL-1 receptor antagonist by human monocytes stimulated with *Mycobacterium leprae* and *M. bovis* BCG. *Int J Lepr,* 1993; **61**: 609–617.
[17] Hass R. Retrodifferentiation – an alternative biological pathway in human leukemia cells. *Eur J Cell Biol,* 1993; **58**: 1–11.
[18] Tsuchiya S, Kobayashi Y, Goto Y *et al*. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. *Cancer Res*, 1982; **42**: 1530–1536.
[19] Tsuchiya S, Yamabe M, Yamaguchi Y *et al*. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int J Cancer*, 1980; **26**: 171–176.
[20] Sanches GA, Malik A, Tougne C *et al*. Simplification and standardization of serodiagnostic tests for leprosy based on phenolic glycolipid -I (PG-I) antigen. *Lepr Rev*, 1986; **57**: 83–93.
[21] Bach MA, Wallach D, Flageul B *et al*. Antibodies to phenolic glycolipid-1 and to whole *Mycobacterium leprae* in leprosy patients: evolution during therapy. *Int J Lepr*, 1986; **54**: 256–267.
[22] Silva CL, Faccioli LH, Foss NT. Suppression of human monocyte cytokine release by phenolic glycolipid-I of *Mycobacterium leprae. Int J Lepr,* 1993; **61**: 107–108.
[23] Schwende H, Fitzke E, Ambs P, Dieter P. Differences in the state of differentiation of THP-1 cells induced by phorbol ester and 1,25-dihydroxyvitamin D3. *J Leuk Biol*, 1996; **59**: 555–561.

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Involvement of male genitalia in leprosy

BHUSHAN KUMAR, INDERJEET KAUR, RANJU RAI, SANJAY K. MANDAL & VINOD K. SHARMA
Department of Dermatology, Venereology and Leprology, Postgraduate Institute of Medical Education and Research, Chandigarh-160 012, India

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Summary Four hundred and sixty-seven male patients with leprosy were screened for genital involvement. Genital lesions were observed in 6.6% of all male cases of leprosy. They were seen most frequently in lepromatous leprosy (25.8%), followed by borderline lepromatous (13.3%) and borderline tuberculoid (1.4%) leprosy.

Introduction

Although involvement of the male genitalia, particularly of the gonads, is well known in leprosy, lesions of leprosy are not commonly found on the genital skin and there have been only a few reports of such involvement.[1] We screened 467 male patients with various types of leprosy to assess the incidence of lesions on the genitalia.

Materials and methods

Four hundred and sixty-seven adult males (new, untreated or under treatment) attending our Leprosy Clinic during the period March 1993 to July 1999 were screened for genital lesions. Detailed history, clinical examination, slit-skin smears and skin biopsies were carried out in all patients as part of the routine work-up. Patients were classified according to the revised classification proposed by the Indian Association of Leprologists.[2] These patients were also examined carefully for the presence of genital lesions.

As biopsies done initially in six cases did not reveal any special feature different from those seen in the cutaneous lesions, no further biopsies were done. The possibility of all other diseases which could result in genital lesions, including sexually transmitted diseases, was considered and excluded by appropriate tests.

Correspondence to: B. Kumar (e-mail: kumarbhushan@hotmail.com)

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Results

There were 293 (62.7%) patients with borderline tuberculoid (BT) disease, 143 (30.6%) had borderline lepromatous (BL) and 31 (6.6%) had lepromatous (LL) disease. Out of 467 patients, only 31 (6.6%) had lesions on the scrotum or penis either alone or on both the sites. Lesions on external genitalia were present in four out of 293 (1.4%) patients with borderline tuberculoid (BT), 19 out of 143 (13.3%) patients with borderline lepromatous (BL) and eight out of 31 (25.8%) patients with lepromatous (LL) disease (Table 1). Mean age of the affected men was 31 ± 8.5 years and the mean duration of disease was 6.0 ± 2.8 years. Seven patients were in reaction (BT-2, BL-3, LL-2). Five patients (BT-2, BL-3) had type 1 reaction and two patients with LL disease had type 2 reaction.

In BT patients, the lesions were in the form of ill-defined hypopigmented patches and well defined indurated erythematous plaque on the scrotum (Figure 1). Two out of four BT patients with prominent genital lesions showed evidence of type 1 reaction.

Scrotal involvement in the form of poorly to well defined infiltrated papules and plaques was seen in BL patients (Figure 2). Three of the 19 BL patients had type 1 reaction and in these cases the scrotal lesions were more erythematous and oedematous (Figure 3). One BL patient had prepucial lesions leading to non-retractibility of the prepuce. One patient with BL leprosy with histoid lesions had prepucial involvement (Figure 4). Fourteen (73.7%) out of 19 patients with BL leprosy had both scrotal and penile lesions.

In LL patients the involvement was in the form of diffuse infiltration and infiltrated papules and plaques (Figure 5). Two out of eight LL patients with genital lesions had ENL lesions on scrotum, out of which one had necrotic lesions on glans penis (Figure 6). Three out of eight (37.5%) LL patients had penile and scrotal involvement and four (50%) patients had penile involvement alone. One LL patient also had a prepucial lesion with difficulty in retraction.

Discussion

Involvement of genital skin in leprosy has not been studied widely, largely because of the difficulty in examining patients in totality in routine clinical set-ups. Although no part of the skin is immune from invasion by *Mycobacterium leprae*,[3] the scrotal skin has been described as an unusual site for leprosy.[4] *M. leprae* has been found in the dartos muscle even after adequate therapy.[5] It is difficult to believe, however, that *M. leprae* located in the dartos muscle could in any way contribute to the production of lesions on the scrotum. Pandya and

Table 1. Type of disease and site of genital lesions in patients with leprosy

Type of disease	No. of patients	Location of lesions			Total (%)
		Scrotal + penile	Scrotal	Penile	
BT	293	2	2	-	4 (1.4)
BL	143	14	2	3	19 (13.3)
LL	31	3	1	4	8 (25.8)
Total	467				31 (6.6)

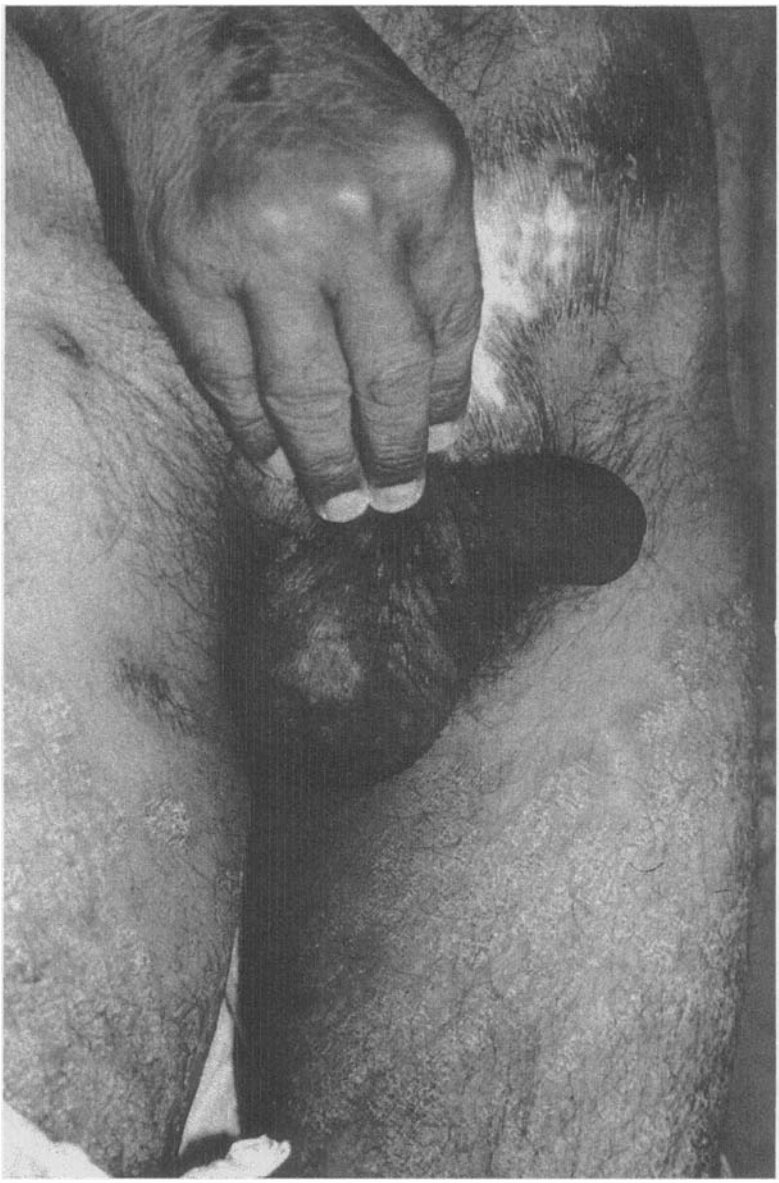

Figure 1. Plaque of BT leprosy in reaction on the scrotum.

Antia[6] have reported leprous granulomas and AFB in one-third of biopsies from the scrotal skin in patients (treated and untreated) with all types of leprosy even in the absence of lesions on the scrotum. According to Anish,[7] dermal infiltrate occurs more in cooler areas of the body than in regions close to the higher core body temperature. Scrotal skin has been reported to be relatively cooler than the core temperature under experimental conditions.[8] However, due to the use of heavy undergarments, it is likely that the temperature of the scrotal skin may

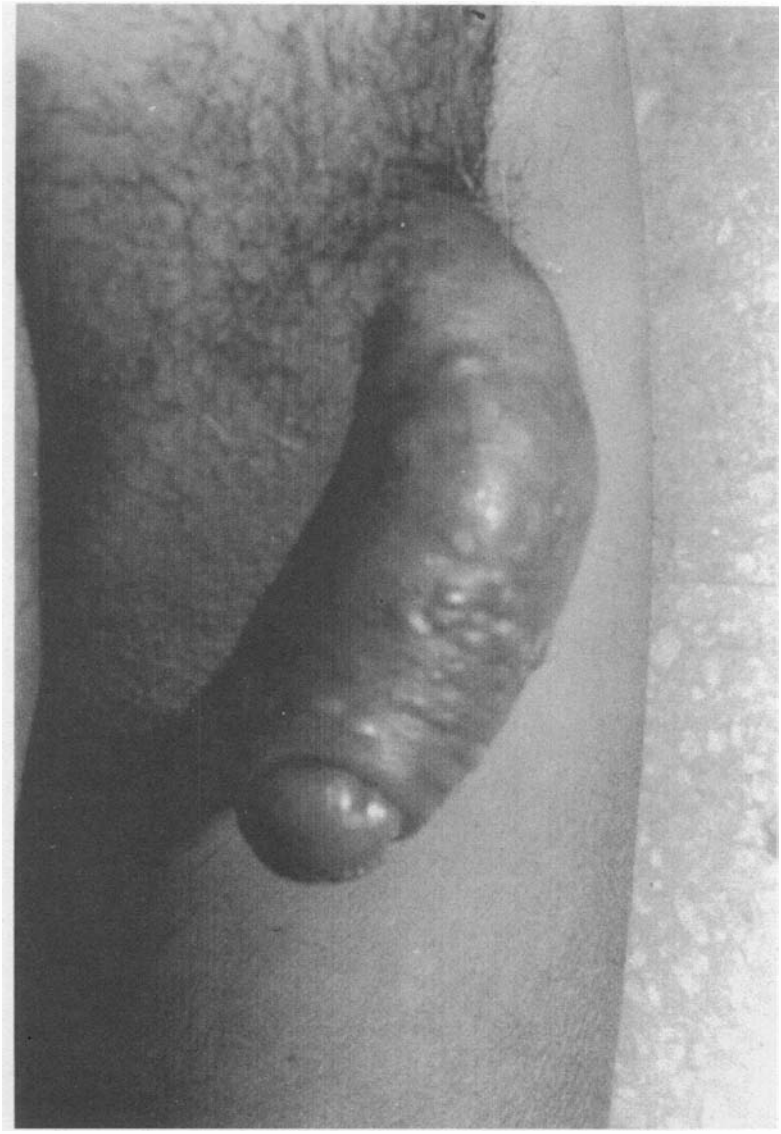

Figure 2. Infiltrated papules over the scrotum in a patient with BL.

remain elevated. Increased temperature may possibly make this area less prone to the development of leprosy lesions.

We have detected lesions in all types of leprosy except TT. Genital involvement in TT patients seems to be rare and we could find only a single case report of this nature in the literature.[9] Arora *et al.*[4] found genital lesions in 2.9% of the cases with borderline disease but did not detect genital lesions in the tuberculoid group. Most of their patients with genital lesions were in the BB group (61.5%), whereas most of our patients belonged to the BL

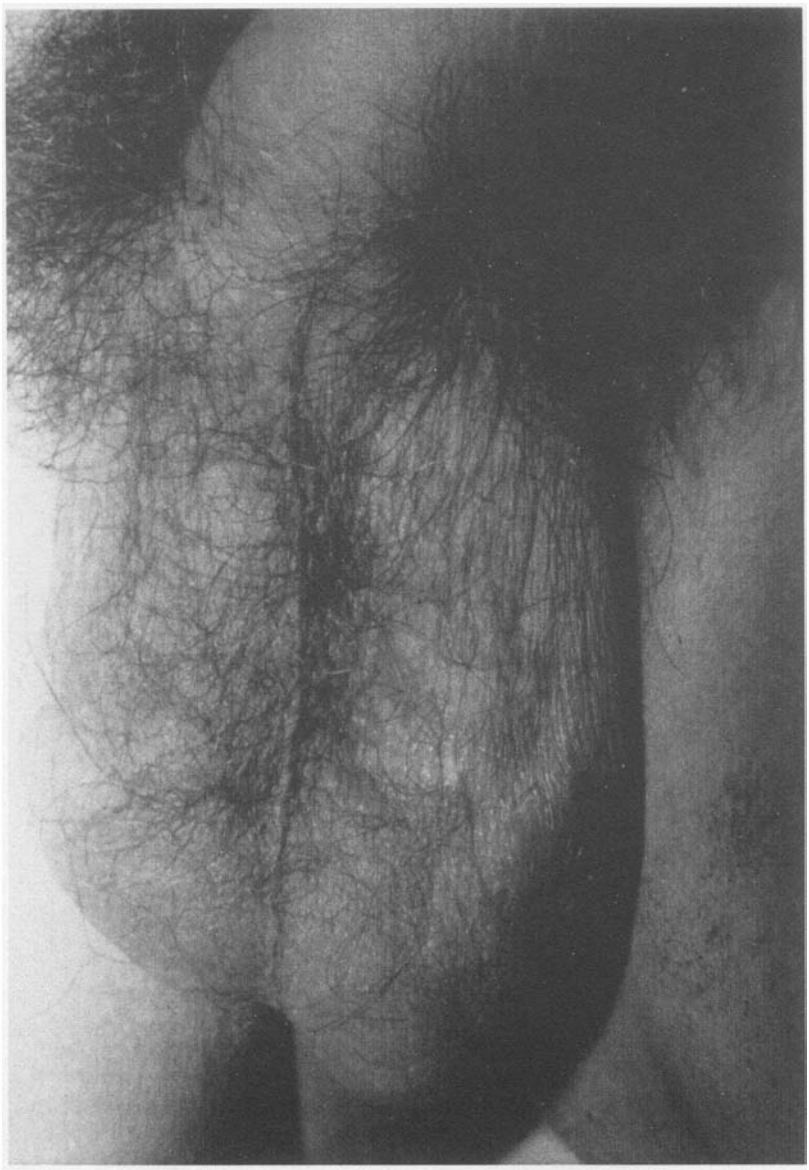

Figure 3. Lesions of BL in reversal reaction on scrotum. Scaly lesions with evidence of subsiding reaction are evident on the thighs.

group. Very rarely, genital lesions in histoid leprosy have also been reported.[10,11] We too found a patient of BL disease with histoid lesions with prepucial involvement.

The incidence of 6.6% for genital lesions in our study indicates that such lesions are not as uncommon as reported previously.[4] They are, however, likely to be missed if not looked for carefully. Their recognition becomes important because genital lesions produced by other diseases may require more serious attention than mere identification.

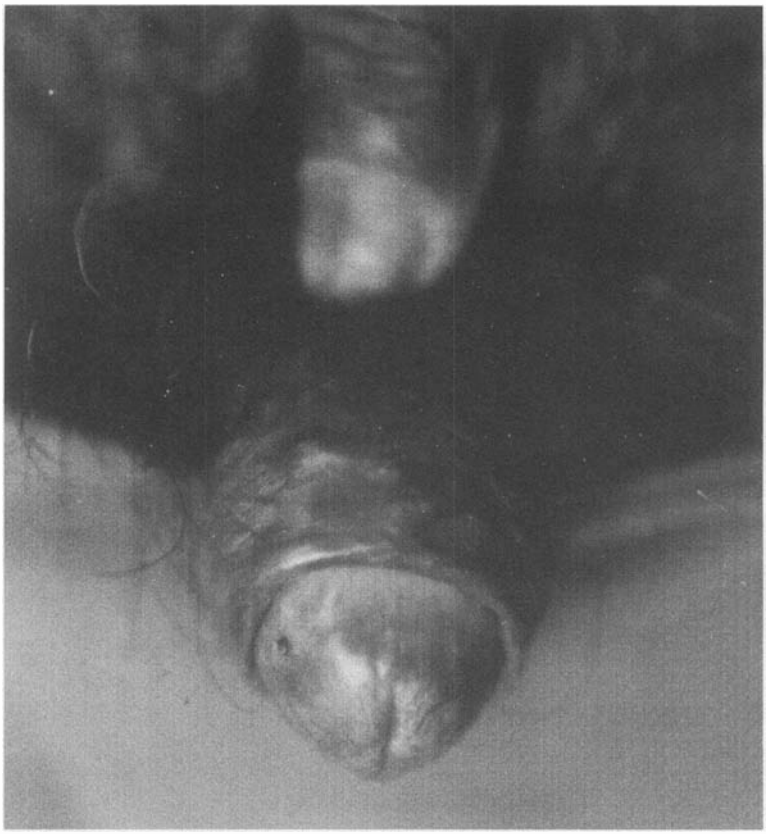

Figure 4. BL leprosy with histoid lesions on the shaft.

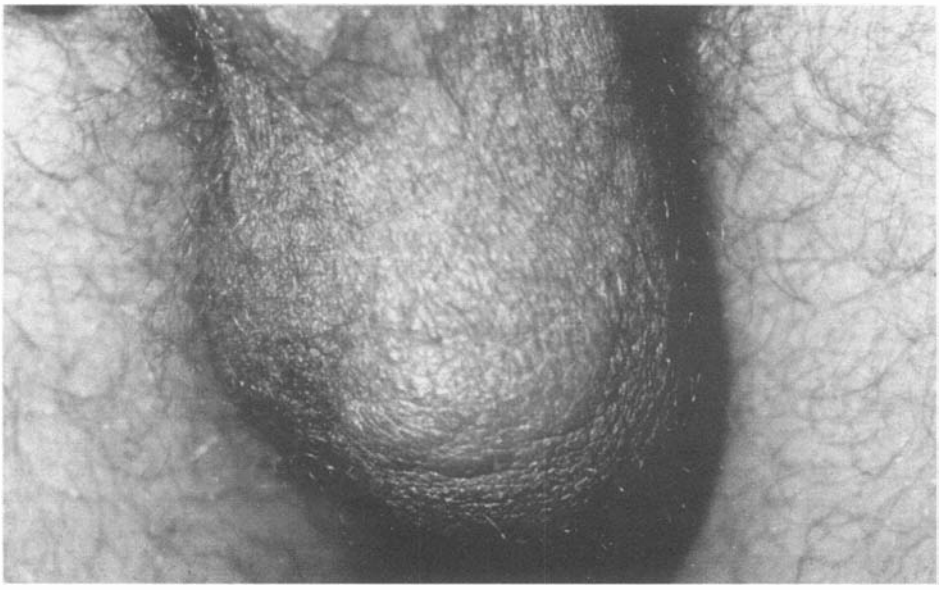

Figure 5. Infiltrated plaque lesion on the scrotum in LL leprosy.

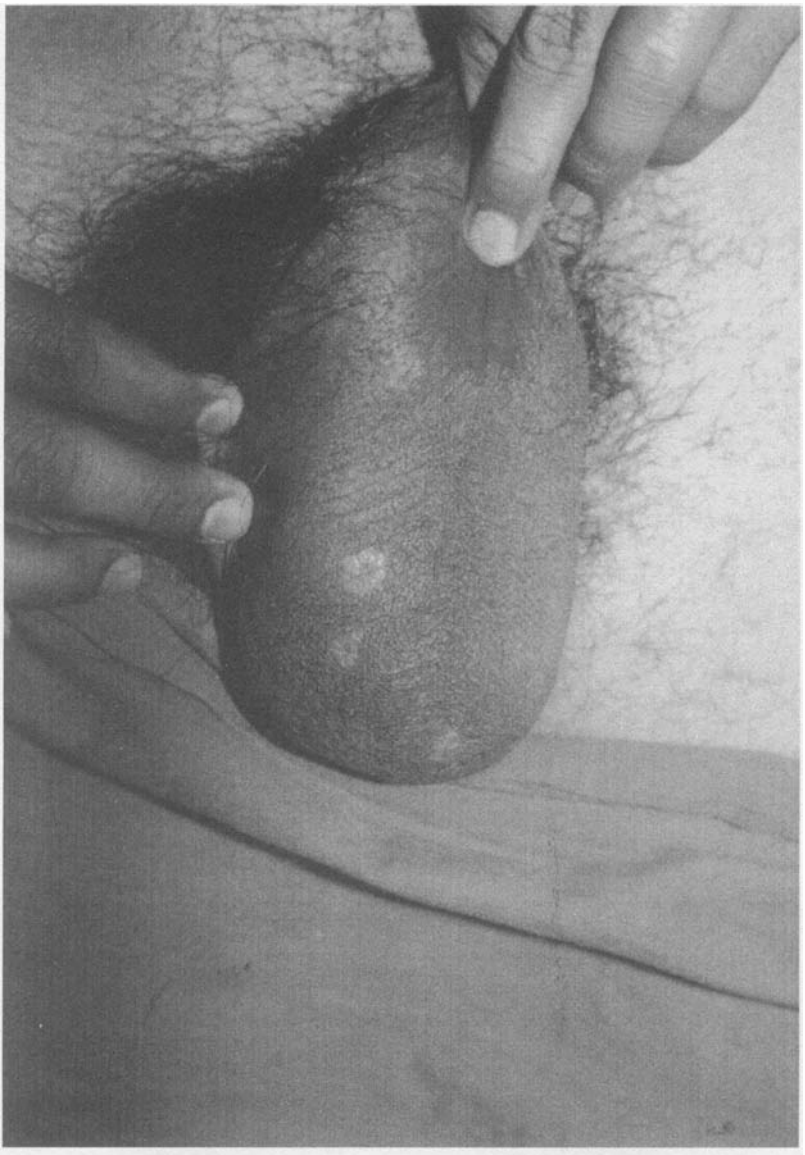

Figure 6. Multiple necrotic ENL lesions on glans penis in LL leprosy.

References

[1] Fox H, Knott J. Leprous nodules of male genitalia. *Int J Lepr*, 1932; **2**: 445–446.
[2] Indian Association of Leprologists consensus classification. *Lepr Ind*, 1982; **54**: 17.
[3] Kaur S, Kumar B. Study of apparently uninvolved skin in leprosy as regards bacillary population at various sites. *Lepr Ind*, 1978; **50**: 38–44.
[4] Arora SK, Mukhija RD, Mohan L *et al.* A study of cutaneous lesions of leprosy on male genitalia. *Ind J Lepr*, 1989; **61**: 222–224.

[5] Ramu G, Desikan KV. A study of scrotal biopsy in subsided cases of lepromatous leprosy. *Lepr Ind*, 1979; **51**: 341–347.
[6] Pandya NJ, Antia NH. The value of scrotal biopsy in leprosy. *Lepr Rev*, 1974; **45**: 145–152.
[7] Anish SA. The relationship between surface temperature and dermal invasion in lepromatous leprosy. *Int J Lepr*, 1971; **39**: 848–851.
[8] Kandeel FR, Swerdloff RS. Role of temperature in spermatogenesis and use of heating as a method of contraception. *Fertil Steril*, 1998; **49**: 1–23.
[9] Dixit VB, Chaudhary SD, Jain VK *et al*. Primary involvement of scrotum in tuberculoid leprosy. *Ind J Lepr*, 1990; **62**: 120–122.
[10] Ramanujam K, Ramu G. Wade's histoid lepromatous leprosy. Report of a clinical study. *Lepr Ind*, 1969; **4**: 293–297.
[11] Nigam PK, Singh G. Mucosal and genital lesions in histoid leprosy. *Int J Dermatol*, 1990; **29**: 207–208.

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Cytodiagnosis of histoid leprosy

A. S. BHAKE*, K. V. DESIKAN** & U. N. JAJOO*
**Mahatma Gandhi Institute of Medical Sciences and **Leprosy Histopathology Centre (LEPRA India), SEVAGRAM-442 102, India*

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Summary This paper presents cytomorphological features of the histoid variety of lepromatous leprosy. Fine needle aspiration of a lepromatous nodule showed cytological features consistent with those of histoid leprosy. Simultaneously, a biopsy of the nodule was also performed and the case confirmed as histoid leprosy. The advantages of the fine needle aspiration technique are that it is simple, quickly reportable, and less traumatizing. Multiple aspirations from different sites may be obtained, which would add to the value of sampling. The need to differentiate a histoid nodule from a conventional lepromatous nodule is explained.

Introduction

Reports on primary cytodiagnosis of leprosy lesions by fine needle aspiration cytology (FNAC) from skin, nerve and lymph nodes are very few in the literature. A few reports of lepromatous tuberculoid leprosy and reactions along with cytomorphological features have appeared in recent literature.[1–4] However, to date, cytodiagnosis of histoid leprosy on FNAC has not been described. The present report describes for the first time the cytomorphological diagnostic features of histoid leprosy by FNAC, confirmed by biopsy.

Materials and methods

A 28 year old man presented himself at the outpatient department with the complaint of nodular swelling on the upper limbs and trunk, which had appeared 3 months earlier. On examination, several nodules 1–1.5 cm diameter were seen on the upper limbs, legs and dorsum of the feet. One nodule on the foot was ulcerated. A nodule on the right forearm and one on the left foot were seen on thickened palpable cutaneous nerves, Skin on back, arms and thighs was oily and shining. There was infiltration on the earlobes. The nodules had a punched-out appearance and were firm. Skin smears were highly positive for AFB, with a BI of 5+, the smears from two nodules being 6+ on the Ridley scale. The clinical diagnosis was of lepromatous leprosy with nodules. A nodule on the right side of neck was selected for FNAC.

*Correspondence to: A. S. Bhake

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The nodule selected for FNAC measured 1 cm × 1 cm and was raised relative to the skin surface. It was situated on the right side of the neck in the mid-lateral position. There was no other swelling or enlarged lymph nodes in the neck. Haematological investigations were within normal limits. There was no fever associated with the nodule.

FNAC was carried out by conventional techniques using a 24 sw disposable needle. The aspirate was thick, white and soft with negligible contamination with blood. A second aspiration was done, with similar results. Four smears were made from each of the aspirates. From each set, two smears were fixed in 95% ethyl alcohol for Papanicolaou staining; one was air dried and stained by Giemsa stain, the other was also air dried and stained by modified Zeihl–Neelson stain.

Findings

The smears showed adequate cellularity. The architectural pattern observed was of two types in the two sets of smears. The first pattern seen in one set of smears was of multilayered cell pallisades of spindle-shaped histocytes on the endothelial vascular core (Figures 1 and 2). Characteristic cells of this pattern showed spindle-shaped cells with round and elliptical blunt nuclei having fine regular chromatin and small nucleoli. There was vesiculation in a few nuclei. Intercellular stroma between these cells was nearly absent.

The second pattern seen in the other set of smears was of isolated cells of polygonal shape with ample cytoplasm (Figure 3). The cells were isolated and in loose groups. These polygonal cell showed abundant cytoplasm which was multivacuolated, presenting a soap bubble appearance. The nuclei in these cells were centrally or eccentrically located. They showed fine chromation with a visible nucleolus. At places there were multinucleated giant cells with the same cytoplasmic character as that of single vacuolated cells. Another characteric feature which was observed in some of the cells was of negative image (intracytoplasmic unstained spaces) seen as small crystalloid spaces in the Giemsa-stained material. The background showed fine and coarse vacuolated material that appeared to be cytoplasmic sheddings. The background also contained negligible lymphocytes.

The smears of both sets stained by the modified Zeihl–Neelsen method showed lntracytoplasmic bundles of acid-fast bacilli.

Histological examination showed a circumscribed granuloma in the dermis, separated from the epidermis by a narrow, clear sub-epidermal zone. The granuloma consisted of thin spindle-shaped cells forming interlacing bands whorls and tight curlicues (Figure 4). Fite's stain showed the cells containing packed acid-fast bacilli with characteristic 'histoid habitus'. Deeper down in the dermis, a classical lepromatous granuloma was seen, composed of sheets of vacuolated macrophages (lepra cells) with moderate infiltration by plasma cells and lymphocytes. These features confirm that this was a case of histoid leprosy and the FNAC findings reflected the histological findings.

Discussion

Cytodiagnosis by FNAC has certain advantages over reporting by excision biopsy. The technique is simple and is less traumatic. As such, it is possible to obtain tissue material from multiple sites and from lesions in different locations that would add to the value of

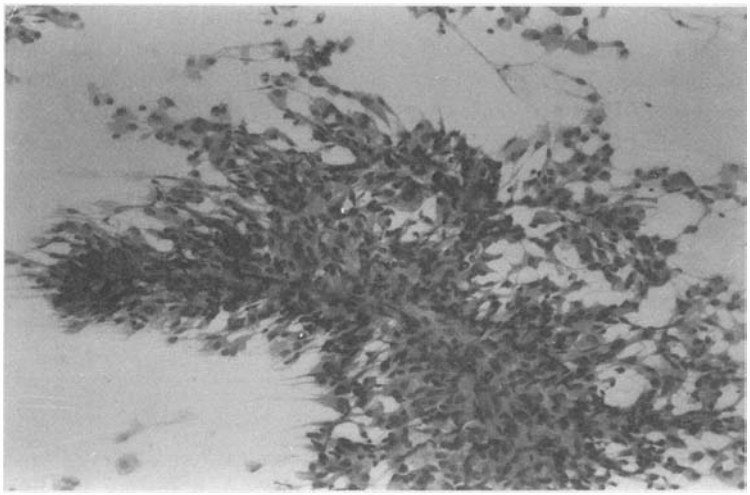

Figure 1. Photomicrograph of FNAC preparation showing multilayered palisades of spindle shaped histocytes. (Papanicolaou × 100).

sampling. Processing the smears, staining and reporting can be done immediately, so that the report is available to the treating physician while the patient waits in the out-patient clinic. Morphologically, the cells could be visualized in more or less their normal shape, since they undergo less shrinkage and distortion. However, FNAC cannot replace excision biopsy where the architecture of the lesion and its relation to other structures can be visualized. Further, by excision biopsy, a larger size of tissue is obtained so that multiple and deeper sections can be obtained.

In the present paper, the cell morphology is clear and evident. The arrangement of cells

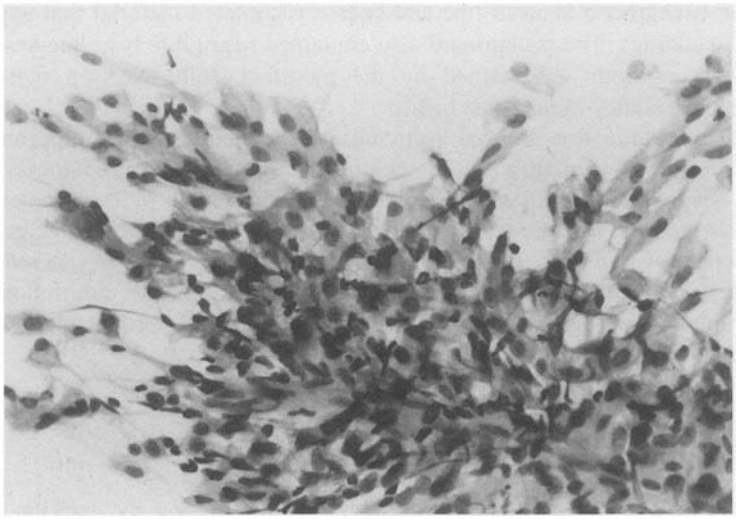

Figure 2. High power view of Figure 1. The spindle shaped cells are broader than those in paraffin sections. Note the endothelial core in the centre. (Papanicolaou × 200).

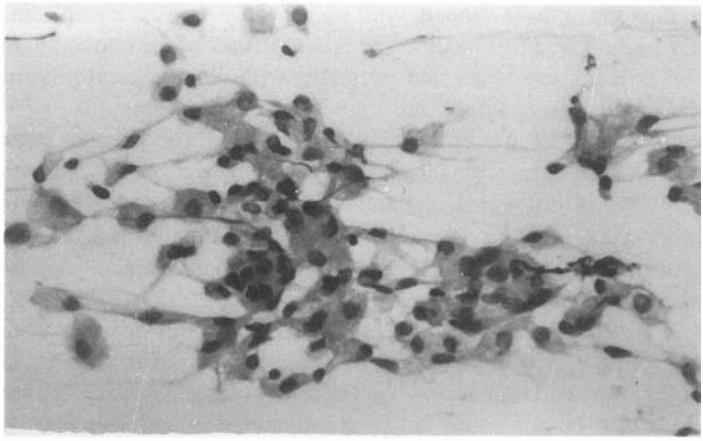

Figure 3. Cells from conventional lepromatous granuloma, irregular or polygonal with abundant cytoplasm. (Papanicolaou × 200).

seen in the smears reflects the architectural pattern of histoid leprosy. Tightly packed spindle shaped cells and forming bands are seen in the Papanicolaou-stained preparation. These cells appear little broader than those in histological sections, and the nuclei are also seen more prominently. An interesting finding in the smears is that the spindle-shaped histocytes are found around an endothelial vascular core (Figure 3), a feature not described in histological sections.

Histoid leprosy was first described by Wade[5] in 1963 to distinguish a relatively unusual form of lepromatous leprosy with characteristic clinical and histological features. The cellular morphology has been subsequently confirmed by several workers.[6–8] Essentially, the lesion is circumscribed, clearly separated by a pseudocapsule from the conventional granuloma of lepromatous leprosy. The cells of the histoid lesion are distinct from the cells of conventional lepromatous granuloma found just adjacent to it. The macrophages in the

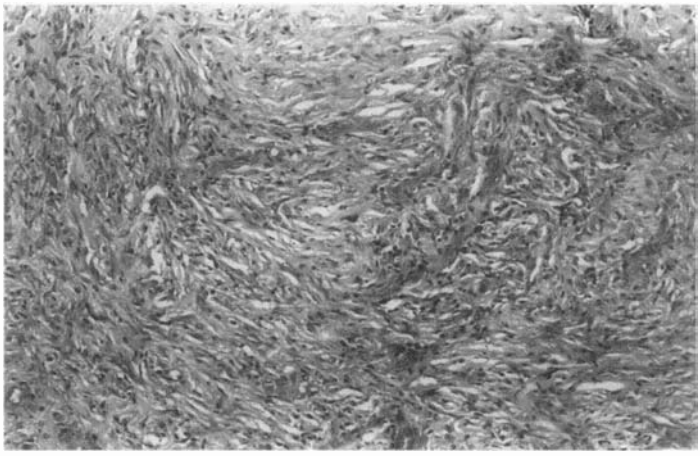

Figure 4. Paraffin section picture showing bands of thin spindle shaped cells forming whorls. (H&E × 200).

histoid type are thin and spindle-shaped, with poor evenly staining cytoplasm. They contain numerous tightly packed acid fast bacilli. The macrophages of the conventional lepromatous lesion, on the other hand, are large, and irregular, with abundant cytoplasm that is highly vacuolated. Acid fast bacilli in 'globi' are found in the cytoplasm.

In the present case, both types of cells have been described. The first pattern with bands of spindle-shaped cells confirmed the lesion to be of the histoid variety. The second pattern of loose or small groups of polygonal, vacuolated cells obviously comes from the adjacent lesion of conventional lepromatous leprosy. It has been pointed out that these cells were mostly found in the second aspiration. It seems likely that on repeated aspiration, the needle must have hit the conventional lepromatous granuloma.

The origin of histoid leprosy is not clear. Job *et al.*[9] consider that histoid lesion is a result of alteration in the growth pattern of *Mycobacterium leprae*, possibly due to loss of immunity in localized areas. Desikan and lyer[7] consider on the basis of morphological criteria that histoid lesions may be borderline between inflammation and neoplasm, but evidence is in favour of an inflammatory granuloma. Nevertheless, histoid leprosy presents interesting clinical and morphological features. It is therefore necessary to recognize and carefully follow up cases of histoid leprosy with regard to drug sensitivity, occurrence of reaction and relapse. Quite often, nodules of lepromatous leprosy are confused with histoid nodules. The distinction is only on cellular morphology. Cytodiagnosis by FNAC is therefore an easy tool to recognize the histoid variety of lepromatous leprosy. However, for management and follow up, skin smear examination would be a better indicator of bacterial load, since it is an accepted tool, needs less expertise and gives a better overall picture of changes in bacterial index.

References

[1] Gupta SR, Kumar B, Kaur S. Aspiration cytology of lymph nodes in leprosy. *Int J Lepr*, 1981; **49:** 9–15.

[2] Cavett JR, MacAtee R, Ramsy I. Hanson's disease (leprosy)—diagnosis by aspiration biopsy of lymph nodes *Acta Cytol*, 1966; **30:** 189–193.

[3] Singh H, Bhatia A, Arora VK, Bhattacharya SM. Fine needle aspiration cytology of lepromatous leprosy. *Lepr Rev*, 1998; **69:** 145–150.

[4] Malik A, Bhatia A, Singh H *et al*. Fine needle aspiration cytology of reaction in leprosy. *Acta Cytol*, 1999; **43:** 771–776.

[5] Wade HW. The histoid variety of lepromatous leprosy. *Int J Lepr*, 1963; **31:** 129–142.

[6] Mansfield R. Histoid leprosy. *Arch Pathol*, 1963; **87:** 580–585.

[7] Desikan KV, lyer CGS. Histoid variety of lepromatous leprosy – a histopathologic study. *Int J Lepr*, 1972; **40:** 149–156.

[8] Girdhar A, Lavania RK, Malaviya GH, Girdhar BK. Histoid lesion in nerve of lepromatous patient. *Lepr Rev*, 1990; **61:** 237–241.

[9] Job CK, Chacko CJG, Taylor PH. Electron microscopic study of histoid leprosy with special reference to histogenesis. *Lepr Ind*, 1977; **49:** 467–471.

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CASE REPORT

Clinical, histopathological and bacteriological investigations in two cases of relapse following ROM treatment

V. P. SHETTY*, A. V. WAKADE*, N. H. ANTIA*, V. V. PAI**, C. R. REVENKER**, R. GANAPATI** & H.O. BULCHAND**

**The Foundation for Medical Research, 84-A R. G. Thadani Marg, Worli, Bombay 400 018, India*

***The Bombay Leprosy Project, Vidnyan Bhavan, 11 V. N. Purav Marg, Sion-Chunabhatti, Bombay 400 022, India*

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Introduction

The efficacy of a single dose of a combination consisting of rifampicin 600 mg, ofloxacin 400 mg and minocycline 100 mg (ROM) for the treatment of single skin lesion paucibacillary (SSL-PB) leprosy has been field tested through multicentre trials in India.[1] This regimen has been accepted and implemented by the Government of India for single lesion cases of leprosy since July 1997,[2] despite some doubts.[3] Further use of three, once-monthly dose of ROM was recommended for cases presenting with two to five lesions.

We report here clinical, bacteriological and histopathological findings in two cases, referred with recurrent lesions following release from ROM treatment.

Case 1

Case 1 (GR), a 40-year-old male, mill worker, was referred to The Foundation for Medical Research (FMR) 2 years after a single dose of ROM treatment. In December 1995, the patient presented with a single coin-sized hypopigmented patch on the right cheek, of 2 months duration. Slit skin smears were negative. He was given a single dose of ROM treatment. The patient was given B complex thereafter. Clinically the patch remained static. In November 1997, there was an increase in the size of the patch, followed by inflammation of the right eye. In January 1998, a punch biopsy was obtained for histopathology and was reported as showing borderline tuberculoid (BT) pathology and the presence of a few acid-fast bacilli (AFB) graded as 1+ in the section. In May of the same year, the patient was referred to FMR

Correspondence to: V. P. Shetty (e-mail: frchbom@bom2.vsnl.net.in, Tel: +91-22-4934989, Fax: +91-22-4932876

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for further investigations. On examination, there was a single large hypopigmented, well-defined anaesthetic patch on the right cheek extending up to the lower eyelid. The right eye was inflamed and had diminished corneal sensation. There was no other evidence of any other patches or involvement of major nerves.

Lepromin was given, but slit skin smears were not performed. A deep incision biopsy was obtained from the patch under local anaesthesia. Biopsied tissue was divided into two parts and studied as follows. One part was fixed in Formol Zenker and embedded in paraffin for light microscopy. The second part was homogenized to determine the bacterial load per g. The homogenate thus obtained was injected into the hind footpads of 20 normal S/W mice to determine the viability and sensitivity to rifampicin. Inoculum size used was 0·03 ml, containing not more than 1×10^4 *Mycobacterium leprae* per footpad. Ten mice were given 0·03 g% rifampicin through mash feed from day 0 (continuous method[4]), and the remaining 10 mice were maintained without any drug as controls. The rifampicin-containing mouse feed was prepared daily in the laboratory. Crushed wheat, milk powder and other essential ingredients were mixed and cooked. The required quantity of drug was mixed into the prepared mash at room temperature using a blender.

In the control group, footpad harvestings were done at months 6, 7 and 8. Two per footpad counts were obtained each time. All the remaining mice were harvested at 1 year. The drug-treated mice were taken up for harvesting, on obtaining a significant fold increase in the control mice or at the end of 1 year.[4]

RESULTS

The lepromin test was negative. Histopathology revealed partial straightening of the rete ridges and the presence of a clear subepidermal zone. Diffused infiltrating cells were seen along the superficial dermis. In the mid- and deep dermis, the infiltrating cells were mostly seen coursing along the adnexa. Several nerve bundles were enlarged and infiltrated both within and around. The infiltrating cells consisted of a mixture of lymphocytes, macrophages that showed mild foamy changes and few epithelioid type macrophages. AFB were seen occasionally in the macrophages and in the nerves. Anti-BCG staining also scored positive (2+). A diagnosis of active BB-BL type of leprosy was made.

The homogenate of the above biopsy also scored positive for AFB. The load was $7{\cdot}55 \times 10^5$/g. initially.

Viability and drug sensitivity

In control mice harvests carried out at post-inoculation months 6, 7 and 8 (two per footpad counts) showed no detectable *M. leprae*. A significant increase ($6 \pm 0{\cdot}3 \times 10^6$ fold) in all four footpads (four mice) were obtained at 1 year. The RFP-treated mice, a total of 10 harvests done at 1 year, showed no detectable bacilli, suggesting sensitivity to RFP at the 0·03 g% level.

A diagnosis of relapse and probable downgrading was made, probably due to ineffective/inadequate treatment. His strain of *M. leprae* remained sensitive to rifampicin.

Follow-up

The patient was retreated in May 1999 with a single dose of ROM. The lesion showed no

change and was raised and erythematous. Bacteriological index in May 1999 was 2+. Thereafter the patient was put on WHO multidrug therapy for multibacillary leprosy.

Case 2

Case 2 (SK) was a 50-year-old housewife who was referred to FMR 19 months after ROM treatment with the development of new skin lesions. In June 1996, the patient presented with more than five hypopigmented, anaesthetic patches on the hands and leg. Slit skin smears were negative. She was given three once-monthly supervised doses of ROM treatment. She developed two episodes of type 1 reaction, which were managed with steroids. In April 1998, she reported back with several new lesions and was referred to the FMR. On examination, the new lesions on the face were raised and erythematous. The patches on the back were hypopigmented, both well and ill-defined. The old patches on the hand and leg were mostly dry and ill-defined. All the patches were anaesthetic. There were also large areas of anaesthesia on the dorsum of both the feet. There were no definite palpable tender or thickened nerves. Lepromin test and multiple smears were done. Two of the patches, one old patch from the right forearm and one new patch from the back, were biopsied using local anaesthesia. Parts of the biopsy were processed for histopathology and homogenization for viability and drug sensitivity tests using the standard mouse footpad method as described earlier.

RESULTS

The slit skin smears were negative. The lepromin was moderately positive (5×4 mm).

Histopathology

The old patch from the forearm showed a mixture of lymphocytes and oedematous epithelioid cells in the superficial dermis at places touching the epidermal layer. In the mid- and deep dermis, the granuloma was active, more organized and localized around the adnexa. A large number of nerve bundles were also seen that showed infiltration both within and around. The infiltrating cells consisted of a large number of epithelioid type of macrophages and few giant cells surrounded by cuff of lymphocytes. The lesion was diagnosed as active BT leprosy.

In contrast, the biopsy from the new patch on the back revealed a small number of aggregates of poorly defined epithelioid cells surrounded by scanty lymphocytes diagnosed as an indeterminate leprosy. In the Trichrome modified Fite-Faracco (TRIFF) stained sections, intact acid fast bacilli were not seen in either of the lesions. Immunostaining using anti-BCG by the PAP method[5] showed the presence of distinct intracellular antigen in both the lesions graded as 1+ and 2+, respectively.

The homogenate prepared of the biopsy from the forearm (old patch) showed no detectable bacilli ($< 1 \times 10^4$) whereas the patch from back (new patch) showed a bacterial load of $2{\cdot}9 \times 10^{6}$/g.

Viability and drug sensitivity

Both the above homogenates, on passage into the footpads, failed to show any increase at any of the intervals studied in control as well as RFP (0·03 g%) treated mice. However, in view of

the histopathological findings, which were indistinguishable from any untreated borderline tuberculoid case, this was concluded to be a case of reactivation/relapse, probably due to inadequate treatment.

Follow-up

From May 1998 to October 1998, the patient was put on steroids with clofazimine. Further information is not available.

Discussion

A single dose of ROM for SSL-PB has been widely used. Moreover, it has also been extended to cases with two to five lesions.[2] The two cases presented in this paper are representative examples. The findings in these two cases cast doubts on the efficacy of this regimen. It is evident that in both these cases the treatment received was inadequate. Both cases were diagnosed, treated and monitored by an experienced team of leprologists. In all probability, both would have downgraded subsequently; the clinical as well as the histopathological findings support a downgrading pathology. Patient SK (case 2), who presented with five skin lesions and was smear negative at onset, showed active BT pathology in the old lesion, despite having received three once-monthly doses of ROM, while the new lesion revealed indeterminate pathology and a detectable bacterial count in the homogenate. Lack of *M. leprae* growth in the footpads of normal mice in this case does not rule out relapse or suggest that it is a reactional episode, because not all BT lesions give a positive yield in the footpads of normal mice.

Patient GR (case 1), on the other hand, had presented with a single coin-sized lesion at onset and was smear negative. On relapse, 2 years after release from treatment, the lesion had increased in size and covered the entire cheek. The patient was lepromin negative and the lesion showed a BB-BL pathology. Moreover, the footpad test showed unequivocal growth of *M. leprae*, which was in keeping with BL pathology. In addition, the inocula was sensitive to 0·03 g% rifampicin tested.

These results highlight the importance of obtaining more data in order to judge the true efficacy of ROM treatment even for SSL-PB cases.

Acknowledgement

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References

[1] WHO action programme for the elimination of leprosy: efficacy of single dose multi drug therapy for the treatment of single lesion paucibacillary leprosy. *Ind J Lepr*, 1997; **69:** 121–129.
[2] Government of India guidelines on modified MDT. Government of India, Leprosy Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Nirman Bhavan, New Delhi 110 011, India, 1997.
[3] Lockwood D. Rifampicin, monocycline and ofloxacin (ROM) for single lesions – what is the evidence. Editorial. *Lepr Rev*, 1997; **68:** 299–300.

[4] WHO. *Laboratory techniques for leprosy.* WHO/CDS/LEP, 1987, 86·4.
[5] Sternberger LA, Hardy PH, Cuculis JJ, Mayer HG. The unlabelled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase antihorseradish peroxidase) and its use in the identification of spirocetes. *J Histochem Cytochem*, 1970; **18:** 315–333.

Lepr Rev (2001) **72**, 88–89

SPECIAL REPORT

Asian Leprosy Congress

The Asian Leprosy Congress was held in the Jaypee Palace Hotel at Agra, India during 9–13 November 2000. This congress was supported by Sasakawa Memorial Health Foundation and the Government of India and co-sponsored by WHO and ILEP. Although this meeting was intended to focus on leprosy problems of Asian countries, it was more or less an international gathering, attended by 1377 delegates from 39 countries, from all over the world.

This Congress was special in the sense that for the first time in the region of South East Asia a large number of workers, from paramedical workers to district and state level officers actively involved in the programme, participated in a forum to share their experiences in the form of scientific papers and in other ways. The congress extended sponsorship to 270 delegates, including 158 participants deputed by the central and provincial governments of several countries.

The prestigious meeting was inaugurated by Mr Sarad Yadav, Honourable Minister of Civil Aviation, Government of India. Mr Yadav made a clarion call to double efforts for elimination of leprosy before the extended deadline of 2005. He recalled the concern Mahatma Gandhi had shown for leprosy patients, especially his personal care to Mr Pasteur Sastri, one of his co-workers, whom he sheltered with him and whose foot ulcer he dressed himself. This exemplary gesture from the Mahatma did much to eliminate the age-old stigma attached to the disease. Dr Yo Yuasa, President, International Leprosy Association, under the auspices of which this Congress was organized, stated that the fight against leprosy, which started in the mid-1940s with promin, continued with dapsone and culminated in the 1980s with the more effective MDT, has been fairly successful. Due to MDT, the millenia-long struggle is approaching an important milestone of achieving elimination by the year 2005. Ahead of that would be the goal of reaching a world without the residual problems, both medical and social.

Dr D. Daumerie, from WHO, expressed the view that MDT implementation close to the community would solve almost all leprosy problems. In his opinion, the real challenge was to spend the next 3 years working more closely with the community, implementing the programme properly and investigating further the strategy in states where the PR is high.

Mr Yohie Sasakawa, President of The Nippon Foundation, Japan emphasized that the technical expertise and total support of WHO, ILEP, NGOs and the administrative heads of leprosy endemic countries had resulted in the treatment of all newly detected patients. The task, he said, was not yet complete, particularly in the South Asian Region, and the Congress in India was appropriate and timely.

Dr S. K. Noordeen, the Chairman of the Congress, stated that this conference was the first in the field of leprosy to focus attention in the region and was being held at an important juncture in the fight against leprosy. Hence, efforts had been made to bring to this forum as many field workers as possible, as they generally received little reward for their efforts. He

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asserted that WHO is playing a critical role by providing effective technical support and leadership in mobilizing national political commitment for leprosy elimination.

Dr C. S. Walter, Organizing Secretary of the Congress, while proposing a vote of thanks, expressed the hope that the problems posed by leprosy would soon disappear due to the the renewed efforts of all delegates after their enthusiasm had been boosted at the Congress.

The scientific programmes consisted of three Panel discussions, eight State of the Art lectures and oral and poster presentations. The first panel discussion was on Elimination of Leprosy and was chaired by Dr S. K. Noordeen. The second and third issues were Immunodiagnostic Tools and Plantar Ulcer and were chaired by Drs P. Brennan and D. D. Palande, respectively. Experts in the respective fields made brief presentations on problematic areas of these subjects, which were then followed by interesting discussions.

The subjects identified for the state of the art lectures were issues of particular interest in terms of control/elimination of leprosy. The titles, speakers and chairpersons of the plenary lectures are given below:

Lecture no.	Subject	Speaker	Chairperson
1	Leprosy elimination	Dr M. Neira	Dr Yo Yuasa
2	Vaccine against leprosy	Dr M.D. Gupte	Dr W. M. Meyers
3	Newer drugs	Dr J. H. Grosset	Dr B. K. Girdhar
4	Nerve damage	Dr C. K. Job	Dr B. Naafs
5	Ocular leprosy	Dr M. Hogewag	Dr P. S. S. Rao
6	Immunopathology	Dr U. Sengupta	Dr K. V. Desikan
7	Social and rehabilitation aspects	Dr S. D. Gokhale	Mr. T. Vasey
8	International classification of disability	Dr W. H. van Brakel	Mr. T. Vasey

Abstracts for as many as 420 scientific papers were received in the Congress. Of these, 288 were accommodated as oral presentations and the remaining 132 as posters. Most papers were on issues related to control and elimination (20%), disability (15%) and chemotherapy and clinical aspects (12%). These papers generally presented success stories of LEC and FDT, including ROM therapy, which has been extended to cases with three lesions for study purposes. Papers related to basic sciences made up only 8% of the total abstracts, but they highlighted important developments in immunology, molecular biology, pathology and microbiology. In addition, the congress generously accommodated views from social scientists and representatives of organizations such as IDEA.

Countrywise, as many as 268 papers were from the host country, followed by China 51, Nepal 32, Brazil 11 and Bangladesh 10. The presentations from China reported mainly post-elimination data, and were of great interest.

Despite the minor chaos of the first day in handling such a large number of delegates, the arrangements generally went well. The overall good rating given by the delegates on the organization of the Congress indicates that as usual they were generous enough to forget the shortcomings and inconveniences. I hope that all the delegates have returned with a sweet souvenir of their stay in the city of Taj Mahal.

D. Poricha
Member, Organizing Committee

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SPECIAL REPORT

Leprosy Review Questionnaire

In March 2000, a questionnaire was circulated to readers of *Leprosy Review*. The questionnaire was intended to give our readers the opportunity to comment on the future direction of the journal. A total of 72 replies were received, and the results are briefly summarized below. A full breakdown of the results is available by e-mail (sboobis@aol.com) on request. Thanks to all those who submitted replies; we are still receiving and analysing them, so it is not too late!

Results

Most of the respondents were aged under 50 years, although eight were aged over 70 years. Approximately 25% of respondents came from the Indian subcontinent, with large numbers also from Nepal and the UK. Most worked in government leprosy programmes, leprosy NGO programmes, leprosy and TB programmes, research and training institutions. They included 32 clinicians, 22 in management/administration, 12 research scientists and 10 teachers, with small numbers in other categories.

There was an extensive list of qualifications, too many to mention here, but medical degrees constituted the majority; 14 respondents had MSc degrees.

The majority of replies were in favour of continuing to publish *Leprosy Review* in its present form. Readers wanted an increase in the number of articles on clinical management, but generally did not feel that there should be more emphasis on research.

Approximately 60% of those replying would be willing to pay more for the Journal to ensure that more original papers could be published. Most were keen to ensure that their institution or organization subscribed to *Leprosy Review*, but a relatively small proportion wanted to subscribe personally.

The vast majority of those replying wished to see an extension of free distribution of *Leprosy Review* to workers unable to afford the subscription.

Most of those replying did submit original articles to *Leprosy Review*, and of those who did not, reasons included no direct involvement in leprosy, no time to write articles, and too few cases to write up.

Three-quarters of respondents stated that *Leprosy Review* was their first choice when submitting original articles for publication. Reasons for not choosing *Leprosy Review* included preference for local journals (2), fear of rejection (2), subject matter too leprosy-orientated (3) and time taken to publish too long (2).

Twelve respondents out of 65 had no access to the Internet; of these, only three did not expect to gain access in the near future. Over 75% of respondents would like to see *Leprosy Review* published in its entirety on the Lepra website, but in conjunction with the printed publication; only 12 out of 43 respondents wanted the material on the website only.

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Almost three times as many respondents, respectively, had access to their copy of *Leprosy Review* through their library or institution, or received it free of charge; only 12 of the respondents had a personal subscription to the Journal.

All the respondents read *Leprosy Review* regularly. The most useful features appeared to be Editorials, Original Articles, Reviews and Case Reports, although it should be said that none of the categories stood out as being particularly unpopular. Of the subject categories, Clinical studies, Epidemiology, Chemotherapy and drug resistance and Prevention of impairment and disability were most popular.

Large numbers of respondents also read the *International Journal of Leprosy, Indian Journal of Leprosy*, ILEP Newsflash/Medical Bulletin and WHO/TDR News, in addition to a wide variety of other journals. The *British Medical Journal* and the *Lancet* were the journals that most respondents also had access to (15 and 9, respectively), although there was a wide variety of both local and international journals. Seven respondents had published in the *International Journal of Leprosy*. Others were generally local or specialist journals, such as *Archives of Ophthalmology*.

The most common reason for publishing in other journals was subject matter, or for variety. Only one had previously been rejected by *Leprosy Review*, and four cited local interest as their reason for favouring other journals.

Conclusions

Respondents appreciated the clinical content of *Leprosy Review*, and in general did not want any dramatic changes made to content. They were keen to have access to *Leprosy Review* on the Lepra website, but not at the expense of the 'paper' journal. There was also a feeling that free distribution of *Leprosy Review* should be increased. It is very encouraging that so many readers find *Leprosy Review* useful in their work, and the Editorial Board will continue to work to improve the service that we provide.

Susan Boobis
(Assistant Editor, Leprosy Review)

Postscript. In January of the year, *Leprosy Review*'s Editorial Board voted to publish each issue of the journal on the LEPRA website (http://www.lepra.org.uk). Readers will now be able to access papers of interest on the website.

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SPECIAL EDUCATION SERIES

An overview of the global epidemiology of HIV/AIDS

HELEN A. WEISS* & SARAH HAWKES**
**MRC Tropical Epidemiology Group, London School of Hygiene & Tropical Medicine, UK*
***Population Council, South and East Asia Regional Office, New Delhi, India*

Summary Over 5 million people continue to be newly infected with HIV every year, despite advances in understanding the factors that drive the epidemics. It is apparent that control of the HIV epidemics has often proved difficult due to the complex web of behavioural, biological, social and structural vulnerabilities to infection. In this paper we discuss the epidemiology and control of HIV in sub-Saharan Africa, and draw parallels with the emerging epidemic in South and South-East Asia. Prevalence of infection in sub-Saharan Africa has continued to increase overall, but a few countries have successfully reduced national infection rates by employing an integrated, multisectoral control strategy. Prevention of similar devastating epidemics in other regions will rely upon an openness in recognizing risk and upon a concerted multisectoral approach to reduction of riskof risk at the individual level andlevel and vulnerability at the societal and structural levels.

Introduction

In the past 2 decades, the human immunodeficiency virus (HIV) has rampaged across the globe leaving virtually no country untouched. Despite advances in our understanding of the social, behavioural and biological factors that directly increase the risk of HIV transmission, approximately 14,500 individuals are infected daily.[1] This high rate of infection has remained constant in recent years, reflecting the difficulties in translating knowledge into better public health.

Over the last 20 years, sub-Saharan Africa has been, and continues to be, the worst affected region, home to 34 million adults and children living with HIV/AIDS, with devastating effects on all aspects of society from family life to international security. To date, no other regions of the world have witnessed a similar degree of devastation, but this may be a temporary phenomenon as the epidemic has arrived relatively recently in

Correspondence to: H. A. Weiss (e-mail: helen.weiss@lshtm.ac.uk, Tel: +44-207-6127872, Fax: +44-207-6368739)

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some parts of the world. In this paper we explore the epidemiology of HIV in sub-Saharan Africa, and draw parallels with the emerging epidemic in South and South-East Asia, which is already home to an estimated 5·8 million adults and children living with HIV/AIDS.[1]

Factors influencing the spread of HIV

In many parts of the world, the predominant mode of transmission has always been heterosexual contact. However, the rates of HIV seen in different geographical settings vary widely as the result of a complex interplay of behavioural, biological, social and structural risks (direct determinants) and vulnerabilities (i.e. factors which may not be directly linked to the transmission of the virus, but may increase the chances of the virus spreading in a particular population); see Table 1.

At an individual level, vulnerability to HIV infection depends on factors that influence the risk of exposure to the virus, such as rate of partner change or frequency of sexual intercourse with an infected partner, and factors which affect the risk of transmission of the virus, such as condom use or presence of another sexually transmitted infection (STI). Many social and demographic factors also influence the epidemiology of HIV (Table 1). High rates of population mobility or urbanization tend to create groups who have an increased vulnerability to infection. Settings where women have not achieved social and economic equality with men also tend to enhance vulnerability, leading, for example, to women's weaker positions in negotiating safer sex (use of condoms), and women resorting to prostitution in the absence of employment opportunities in more formal sectors.

The interplay of these factors can have a dramatic effect on infection rates. Figure 1 shows HIV prevalence among teenage girls in Kisumu, western Kenya. Almost 30% of girls aged 17 were HIV positive compared with only 4% of boys of a similar age. This striking difference suggests that young girls are particularly susceptible to infection, through having unprotected sex with older, infected, men and perhaps due to starting sex at a very young age.[2]

Table 1. Factors influencing risk and vulnerability to HIV

Individual factors	Social and demographic factors	Structural factors
Protective behaviours (e.g. use of condoms)	Age structure of population	Position of women
Type of partnerships	Sexual mixing patterns	Wealth of population
Injecting drug use	Population mobility	Income distribution
Lack of knowledge	Rates of urbanization	Policy environment
Denial of risk	Gender-based education rates	
Age	Rates of male circumcision	
Gender	Access to effective STI treatment	
Presence of other sexually transmitted infections (STIs)	Availability and use of commercial sex	
Male circumcision status	Services for drug users	
	Safety of blood transfusion	

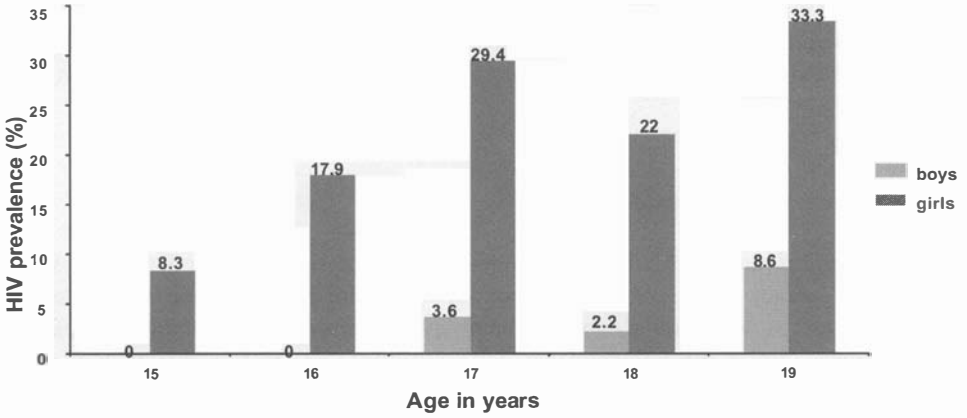


Figure 1. HIV prevalence rate among teenagers in Kisumu, Kenya, by age (source: National AIDS Programme, Kenya, and Population Council, 1999).

Epidemiology of HIV in sub-Saharan Africa

Sub-Saharan Africa provides ample evidence of differences in prevalence, with most countries in West Africa consistently reporting lower rates than those in Eastern or Southern Africa. To explore the relative importance of the factors listed in Table 1, one recent study compared reported sexual behaviour with data on biological 'co-factors' in four cities. Two were cities with relatively low, and fairly stable prevalence of HIV (Yaounde, Cameroon and Cotonou, Benin), and two were cities in eastern/southern Africa with very high HIV rates – Kisumu, Kenya and Ndola, Zambia.[3] Few differences in sexual behaviour were found, although men and women in the high prevalence cities tended to be younger at first sex and at marriage, features which are likely to have helped drive the epidemics. Biological factors, however, were found to explain more of the variation in HIV rates than differences in sexual behaviour. Other sexually transmitted infections, especially the ones that cause genital ulcers, such as HSV-2, are known to enhance the spread of HIV.[4] HSV-2 infection is highly prevalent in many African countries. Individuals with both infections are at high risk of transmitting both viruses due to the more frequent recurrences of genital herpes, giving rise to a 'reinforcing epidemic'. This might explain in part why the HIV epidemic has spread so rapidly in countries with high levels of HSV-2 and other sexually transmitted infections.

Another factor that may explain the lower prevalence in most West African countries is the protective effect of male circumcision against acquiring HIV. This hypothesis was first postulated in 1987,[5] and the epidemiological evidence for a strong protective effect (a reduction in risk of around 60%) is now compelling.[6] Such a protective effect is biologically plausible,[7] but its translation into public health practice is fraught with difficulties, as male circumcision is often an important cultural or religious practice. Further, there is the concern that men may alter their sexual behaviour if they believe they are protected from infection by circumcision.

Strategies to reduce heterosexual transmission of HIV

In the absence of a vaccine against HIV, current prevention strategies focus on changing

sexual behaviour and on creating a social environment that reduces a population's vulnerability to the epidemic. Such strategies include promoting the use of condoms, directing STI treatment programmes at specific populations that are at high risk, community-wide programmes to reduce risk, education in schools, and voluntary counselling and testing. A recent review showed that many trials of such interventions in communities in developing countries were successful in reducing HIV incidence in the communities where the interventions occurred.[8] For these strategies to reduce national rates of HIV, however, the combined efforts of the Government and non-Governmental groups are needed, including religious leaders and community development organizations working together to inform and educate the population. There are still very few examples of this integrated approach. One country which has successfully reduced the number of new infections is Uganda, where prevalence has dropped from 14% in the early 1990s to around 8%, consistent with reports of delayed onset of sexual activity and marriage, and increased condom use.[9] Recent data show that HIV rates may also now be falling in Zambia, one of the worst affected countries, where again there is an integrated approach. HIV rates among pregnant girls aged 15–19 in the capital, Lusaka, have halved from around 25% to 12% in the last 6 years.[2]

Epidemiology of HIV in India, south and south-east Asia

No other continent has experienced the extensive spread of HIV that sub-Saharan Africa is suffering from. In part, this may be a result of the age of the epidemic, a factor known to account for some of the recorded differences in prevalence worldwide.[10] Other areas of the world, however, are now reporting an increasing incidence of HIV. For example, India is currently estimated to have 3.7 million HIV positive persons – up from an estimated 2.7 million in 1998.[11] The first HIV-positive person was identified in India in 1986, and there has since been a rapid spread of the epidemic in several parts of the country. It is estimated that most of these infections have been acquired through sexual transmission (80%), with smaller numbers from injecting drugs (5%) and from infected blood transfusions (5%), and a few through mother to child transmission (<1%).[11]

India, like many other countries, does not have a single HIV epidemic, but has multiple epidemics in different geographical settings and among people with different types of risk. Initial cases were reported among female sex workers in the cities of Mumbai and Chennai, and among injecting drug users in the north-east of the country, especially in the state of Manipur.[12] Since then, however, there has been a diffusion of the epidemic away from recognized 'high risk groups' and into the so-called 'general population'. The seropositivity rate among pregnant women (taken as representative of the sexually active 'general population' of women of reproductive age) in selected sentinel sites ranges between zero and over 2% (in the States of Maharashtra and Karnataka), and is as high as 6% in some areas of slums of the city of Mumbai.[13] Studies which have examined the prevalence of HIV infection in married women have found that husbands' reported behaviour was often the major risk factor.[14–16]

Most other countries in south Asia have lower HIV prevalence rates than those reported in parts of India. However, the region exhibits high levels of risk in most of the factors which are known to increase population vulnerability: gender inequality, poverty and disparities in income distribution, low levels of formal education and literacy, rural-urban divides which

lead to high levels of population movement and high levels of inter-country migration – for example, it estimated that 165,000 men leave Bangladesh each year to work abroad.[17] Given the already high burden of disease, economic poverty and generally poor ranking in tables of global development, the 1·2 billion people who live in the countries of south Asia are at risk of an extended HIV epidemic. The ability of south Asia to prevent such an epidemic will rely upon an openness in recognizing the problem and its challenge, and a concerted multisectoral approach to reduction of individual risk and societal vulnerability.

The south-east Asian epidemic has been well documented in Thailand, where HIV initially spread rapidly in the late 1980s among injecting drug users and between sex workers and their clients. The government acted quickly to set up a comprehensive prevention campaign including enforced condom use in establishments used by sex workers and a mass advertising campaign.[18] HIV rates have now stabilized and the prevalence in the general population is around 2%. Meanwhile, neighbouring Cambodia has suffered one of the highest recorded HIV rates in south-east Asia: in 1998, 3·6% of adults aged 18–45 years were estimated to be HIV positive, including 2·6% of women of childbearing age.[19] The country has one of the highest rankings on the Human Poverty Index in Asia,[20] and a limited infrastructure with which to cope with the impact of HIV disease.

HIV epidemiology in other regions

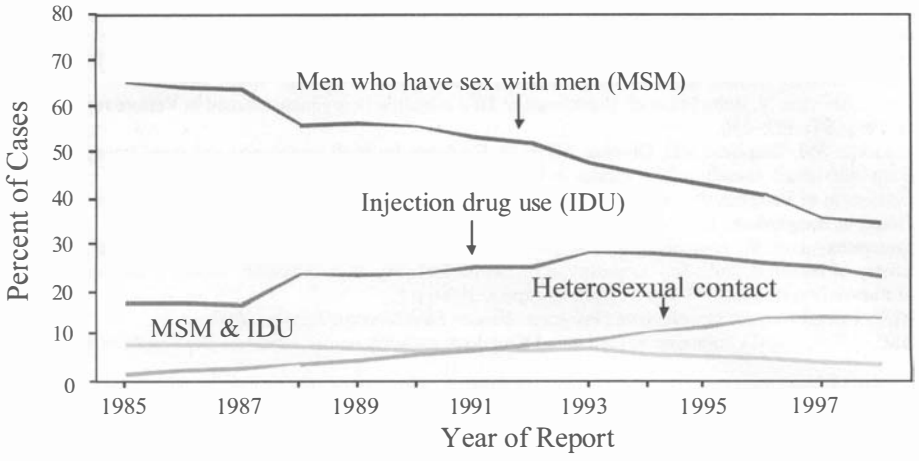
In Europe and the United States of America, the dynamics of the epidemic have been very different, a reflection, perhaps, of these societies' different risks and vulnerabilities (see Table 1 for examples of variables which will differ in prevalence between regions). Mortality due to HIV/AIDS has decreased sharply since the mid-1990s due the availability of anti-retroviral drugs The number of new infections, however, is not decreasing, highlighting the need to maintain effective strategies for prevention. For much of the past 2 decades, the risk of acquiring infection has remained highest among men who had sex with men, and injecting drug users. Now the pattern is changing, with heterosexual intercourse the main mode of transmission in the UK[21] and other countries in northern Europe. A similar trend is seen in the United States (Figure 2).

In the Caribbean too, heterosexual transmission is the cause of most HIV infections, leading to rates in the general population of around 3–5% in several countries in the region, including Haiti, the Bahamas and the Dominican Republic. Up to 10% of pregnant women are estimated to be HIV positive in some urban centres in Haiti. Heterosexual transmission is increasing in Latin America also, where the epidemic was initially concentrated among homosexual men and injecting drug users.

In the year 2000, HIV epidemics have exploded in many countries of Eastern Europe and Central Asia, and the number of infected adults and children has almost doubled to 700,000. The main mode of transmission in this region is injecting drugs. Social and structural factors outlined in Table 1 will shape the epidemic in this region, where socioeconomic instability is already causing an increase in drug use and commercial sex. However, some encouraging strategies for prevention have been put in place.[1]

Conclusions

Thousands of people continue to be newly infected with HIV every day, contributing to the many HIV epidemics in different regions of the world. The diversity of the epidemics results



Excludes cases with other or unreported risk pending medical record review and reclassification.

Figure 2. AIDS cases by exposure category and year of report, 1985–1998, United States (source: Centers for Disease Control & Prevention, National Center for HIV, STD and TB Prevention, Atlanta, Georgia, USA).

from a complex interaction of behavioural, social and biological factors. Sub-Saharan Africa has suffered most, with devastating social and economic consequences. Many of the factors that fuelled the epidemics in Africa are present in other developing countries around the world, and pose a formidable public health challenge. The response to this challenge must be based on the recognition of the diverse risks and vulnerabilities in each population, and must integrate the attitudes and efforts of different sectors of society and the public health authorities.

References

[1] UNAIDS/WHO. *AIDS epidemic update.* UNAIDS, Geneva, December 2000.

[2] UNAIDS/WHO. *Report on the global HIV/AIDS epidemic.* Geneva: UNAIDS, Geneva, June 2000.

[3] Buve. A. for the Study Group on Heterogeneity of HIV Epidemics in African Cities. HIV/AIDS in Africa: Why So Severe, Why So Heterogeneous? *7th Conference on Retroviruses and Opportunistic Infections*, San Francisco, CA, USA, February, February 2000 [Abstract S28].

[4] Fleming S, Wasserheit J. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. *Sex Trans Inf*, 1999; **75:** 13–17.

[5] Fink A. Circumcision and heterosexual transmission of HIV infection to men. *N Engl J Med*, 1987; **316:** 1546–1547.

[6] Weiss HA, Quigley MA, Hayes RJ. Male circumcision and risk of HIV infection in sub-Saharan Africa: a systematic review and meta-analysis. *AIDS*, 2000; **14:** 2361–2370.

[7] Szabo R, Short RV. How does male circumcision protect against HIV infection? *BMJ*, 2000; **320:** 1592–1594.

[8] Merson MH, Dayton JM, O'Reilly K. Effectiveness of HIV prevention interventions in developing countries. *AIDS*, 2000; **14:** S68–84.

[9] Asiimwe-Okiror, G G, Opio A, Musinguzi J *et al*. Change in sexual behaviour and decline in HIV infection among young pregnant women in urban Uganda. *AIDS*, 1997; **11:** 1757–1763.

[10] Over M. The effects of societal variables on urban rates of HIV Infection in developing countries: an exploratory analysis. In: Ainsworth M, Fransen L, Over M (eds) *Confronting AIDS: evidence from the developing world.* European Commission and the World Bank, 1998.

[11] National AIDS Control Organisation. http://www.naco.nic.in/vsnaco/indianscene/update.htm (07/09/00).

[12] Pal SC, Sarkar S, Naik TN *et al*. Explosive epidemic of HIV infection in north-eastern states of India, Manipur and Nagaland. Indian Council for Medical Research. *CARC Calling*, 1990; **3:** 2–6

[13] National AIDS Control Organization. *Country Scenario 1997–98*. Government of India, Ministry of Health and Family Welfare, New Delhi, 1998.
[14] Jacob M, John TJ, George S *et al*. Increasing prevalence of human immunodeficiency virus infection among patients attending a clinic for sexually transmitted diseases. *Ind J Med Res*, 1995; **101:** 6–9.
[15] John TJ, Bhushan N, Babu PG *et al*. Prevalence of HIV infection in pregnant women in Vellore region. *Ind J Med Res*, 1993; **97:** 227–230.
[16] Mehendale SM, Shepherd ME, Divekar AD *et al*. Evidence for high prevalence and rapid transmission of HIV among individuals attending STD clinics in Pune, India. *Ind J Med Res*, 1996; **104:** 327–335.
[17] Government of Bangladesh and UNICEF. Master plan of operations for a programme of services for women and children in Bangladesh, 1993–1995. GOB/UNICEF, Dhaka, 1993.
[18] Rojanapithayakorn W, Hanenberg R. The 100% condom program in Thailand. *AIDS*, 1996; **10:** 1–7.
[19] Ministry of Health (Cambodia). Department of Health Planning & Information, *Health Situation Analysis 1998 and Future Directions 1999–2003* (briefing paper), 1999, p 6.
[20] UNDP. United Nations Development Program, *Human Development Report*, 2000.
[21] CDSC. AIDS and HIV infection in the United Kingdom: monthly report. *Commun Dis Rep CDR Wkly*, 2000; **10:** 157–160.

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Book Review

The care of neuropathic limbs; a practical manual. **Grace Warren**
Parthenon Publishing Group, ISBN 1-85070-048-6, £45 per copy

This book is the distillation of some 40 years experience in managing neuropathic limbs, mainly in those persons affected by leprosy but also in later years in advice to diabetic and orthopaedic specialists in 'Western countries'. For the new doctor in a leprosy control programme seeing a warm swollen ankle joint and an X-ray with minimal changes of dubious significance this is a really helpful book. It gives a clear if somewhat didactic understanding of the aetiology of the slowly inexorable changes of neuropathic bone disintegration, and helps the confused doctor make a rational plan of what to do to prevent the dreadful consequences, if the condition is not actively managed. Such information would also be of considerable interest to physicians responsible for neuropathic joints in developed nations. Here, the management is often relegated to a junior doctor or outpatient nursing service and unless such people are thinking about neuropathy as a cause of the patient's warm swollen joint, the diagnosis will go unnoticed for months if not years and much deformity will develop before any action is taken to control the problem.

The reader who likes evidence will be disappointed, as Grace provides only one reference in the whole manuscript. She makes strong statements about management mostly based on her considerable experience, even though there is already published data for some of her assertions, which may seem strange to specialists not previously working in this field. However, the absence of evidence will cause some to consider her statements to be beliefs rather than facts, especially regarding the process of inflammation and healing in neuropathic joints. This aside, the text provides an excellent framework in order to assess and treat neuropathic injuries and will be essential for any department regularly dealing with potential patients to help them both spot the diagnosis early and then help them with practical advice on how to actually control the problem. There are excellent chapters on 'the significance of hot spots', 'radiology of the lesions' and 'the development of disintegrating bone lesions'.

Those who know Grace will be able to 'hear' her voice in this book, as the style is rather wordy and repeats itself, but only with the purpose of making the points that those seeing potential people affected with a neuropathic joint might not miss these points.

At £45 it is a good buy, and I recommend it to all who might have occasion to treat these patients. After you have read this book, you will probably be the only person who will have any idea as to what is going on with the individual, and you may need to point out some salient feature to a more senior colleague.

Bruce Richard MS FRCS

Grace Warren's book may also be ordered through TALC, at a basic retail price of £12 for small quantities (approximately A4 page size, flexible laminated cover). Available also from The Leprosy Mission, PO Box 293, Box Hill 3128, Victoria, Australia. For price and postage, fax +61 3 9890 0550 or e-mail treenah@leprosymission.org.au.

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Letter to the Editor

'JHUM-JHUM' – A COMMON PARAESTHESIA IN LEPROSY

Editor,

Peripheral neuropathy in leprosy commonly manifests as a progressive loss of sensation and voluntary muscle function. However, at times leprosy patients complain of pain or paraesthesia in leprosy affected limbs. The significance of these symptoms for nerve function impairment is unclear. In Nepal, a syndrome of paraesthesia ('jhum-jhum') has been described.[1] Almost 30% of people from a tribal group in southwest Nepal complained of numbness or tingling in a 'stocking and glove' distribution. Jhum-jhum is also commonly reported in Nepali patients with leprosy, diabetes, vasculitis and hyperventilation and in those treated with isoniazid.[2]

We examined the records of 277 previously untreated patients enrolling for MDT treatment at Anandaban Leprosy Hospital, Kathmandu, Nepal between 1993 and 1995 and who attended the outpatients department regularly for an average of 30 months (range 6–84 months) for recorded episodes of jhum-jhum. In all, 107 (39%) patients had recorded episodes of jhum-jhum. This is almost certainly an underestimate, as patients were not asked if they experienced symptoms; this study depended on the patient reporting and the doctor recording the complaint. Most jhum-jhum was manifested as burning and tingling sensations (47%) or neuropathic pain alone or in combination with burning and tingling (45%). Some patients gave vivid descriptions of altered sensation such as 'ants crawling on my skin'. Symptoms were more commonly reported in the extremities: hands only (25%), feet only (20%), both bands and feet (33%) than in the face (4%) or whole body (1 8%). The majority of patients (72%) complained of jhum-jhum in limbs with leprosy-affected nerve supply as evidenced by enlarged nerve trunks or areas of anaesthesia. Jhum-jhum occurred in leprosy patients before (15%), during (61%) and after (24%) anti-leprosy treatment. About two-thirds of patients appeared to have episodic symptoms of jhum-jhum, while in one-third symptoms persisted for many months. The exact length of time the symptoms persisted could not be determined accurately from our records. Although associations with clinical parameters could not be drawn with confidence from this retrospective study, there was an association between the frequency of reported jhum-jhum and age (Chi-squared test for linearity $= 10$, $P < 0{\cdot}005$). This may reflect an increase of paraesthesia in older persons in general, rather than a specific leprosy association. There was no apparent association with gender or with type of leprosy disease (pauci- or multibacillary) or with the occurrence of either type 1 or ENL reactions.

This high frequency of paraesthesia in leprosy patients is deserving of further attention. This complaint, though distressing to the patient, is generally left untreated by the physician unless there is evidence of nerve function impairment. Although we could find no evidence in this selected group of patients for any association of jhum-jhum with loss of nerve function, the question remains open as to whether these symptoms are associated with loss of function. In the early twentieth century, paraesthesia were noted as a presenting symptom in leprosy patients and this progressed to frank anaesthesia.[3,4] Alteration in sympathetically mediated vasodilation and the sweat response has been documented to parallel degeneration of sensory nerves in leprosy affected limbs.[5] The extent to which leprosy-related paraesthesia is due to autonomic nerve degeneration cannot be commented upon here, since no measurements of jhum-jhum affected patients' autonomic skin or other responses were made. Whatever the cause of persistent neural paraesthesia, the end result physiologically is that spinal dorsal horn

neurons cease giving inhibitory input to primary afferent pain neurons in affected limbs, allowing spontaneous firing of the afferent neurons when there is no stimulation.[6]

Paraesthesia and neuropathic pain is frequently associated with psychopathology. In Nepal, about 25% of adults in a general clinic were shown to have high scores on the WHO SRQ-20 depression scale (Wright, 1988, unpublished observations) and while none presented with psychiatric complaints, all had somatic symptoms including jhum-jhum. Among the Nepali villagers complaining of jhum-jhum, 48% had neurotic symptoms and 62% displayed at least one psychotic symptom.[1] Mental health training programmes in Nepal now teach peripheral health workers to screen all those who present in general clinics with jhum-jhum for signs of depression.[7]

Among leprosy patients, depression may be a significant problem and this may contribute to or be associated with somatic symptoms such as jhum-jhum. The extent of the contribution of psychopathology to neuropathic pain and paraesthesia in leprosy remains to be elucidated. Psychopathology in neuropathic pain syndromes has been documented: in one study of 59 patients with painful diabetic neuropathy; all had elevated depression scale scores, and all had some alleviation of their painful neuropathy on treatment with antidepressants.[8] In an unpublished survey in Nepal, 20% of leprosy patients had an elevated YMO-SRQ20 score (Theuvenet, personal communication).

The differential diagnosis of peripheral paraesthesia-causing neuropathies is vast, and includes other hypovitaminoses (such as deficiency of thiamine, called cerebral beriberi, or of cyanocobalamine), connective tissue diseases such as amyloidosis, chronic poorly controlled diabetes mellitus, toxin ingestion (pesticide organophosphates, hexane and other fat-soluble hydrocarbons, and lead), isoniazid treatment of tuberculosis, metabolic diseases such as porphyria or uraemia, viral syndromes such as herpes zoster, chronic demyelinating diseases and a variety of rare genetic disorders.[9] Of these, possibly only diabetes mellitus and treatment for tuberculosis were likely to have been detected in the Anandaban Leprosy Clinic, since all leprosy patients are screened at least at the time of initial presentation for tuberculosis and diabetes mellitus.

In summary we have evidence that parasthaesia is a common complaint among leprosy patients. There is a need to determine the full extent of the problem and the degree of association with psychiatric illness, particularly depression. The possible link to frank nerve function loss also needs to be established. The ready treatment of neuropathic pain with antidepressants, particularly the tricyclic antidepressants, may be an important palliative treatment option for leprosy patients with jhum-jhum.

Mycobacterial Research Laboratory
Anandaban Leprosy Hospital
Kathmandu, Nepal
(e-mail: roche@umn.mos.com.np)

J. W. LEMASTER, O. JOHN & P. W. ROCHE

References

[1] Kohrt BA, Screiber SS. Jhum-jhum: neuropsychiatric symptoms in a Nepali village. *Lancet*, 1999; **353:** 1070.
[2] Basnyat B, Zimmerman M, Sleggs J, Vaidhya H. Jhum-jhum is a symptom (letter). *Lancet*, 1999; **353:** 2074.
[3] Marchoux E. La lepre. *Rev Hyg*, 1913; **24:** 883–939.
[4] Monrad-Krohn GH. The neurological aspects of leprosy. *Vid Selsk Skr*, 1923; **16:** 78.
[5] Facer P, Mathur R, Pandya SS *et al.* Correlation of quantitative tests of nerve and target organ dysfunction with skin immunohistology in leprosy. *Brain*, 1998; **121:** 2239–2247.
[6] Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms and management. *Lancet*, 1999; **353:** 1959–1964.
[7] Hickingbottom D, Wright C. Training in psychiatry for community health workers in Nepal. *J Nepal Med Assoc*, 1987; **25:** 1–11.
[8] Turkington RW. Depression masquerading as diabetic neuropathy. *JAMA*, 1980; **243:** 1147–1150.
[9] Kumar P, Clark M. *Clinical medicine*, 3rd edn. Saunders, London, 1994, pp 946–949.

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Letter to the Editor

A CRITIQUE ON COMMENTARY 'HOW *MYCOBACTERIUM LEPRAE* INFECTS PERIPHERAL NERVES' BY FREEDMAN *ET AL.*

Sir

Understanding of the mechanism/s of entry of *Mycobacterium leprae* into the peripheral nerve compartment and damage to neural constituents is extremely important in its prevention or modulation. In this context, the recent findings of Rambukkana and co-workers are indeed very important. We read with interest a science commentary 'How *Mycobacterium leprae* infects peripheral nerves' by Freedman and co-workers; that appeared in the June 1999 issue of *Leprosy Review* (**70**: 136–139) and another comment by the same group.[1] The authors postulate the mechanism of nerve infection in leprosy on the basis of recent findings by Rambukkana and coworkers[2,3] which calls for certain clarifications.

The first paper of Rambukkana and colleagues[2] suggested the 'G' domain of the laminin $\alpha 2$ chain as a host-derived bridging molecule, and proposed that *M. leprae* interacts with host Schwann cells by binding to the $\alpha 6\beta 4$ integrin which acts as a laminin receptor on the Schwann cell surface. These primary Schwann cells and Schwann cell neuron co-cultures were devoid of the fibronectin that is normally present in their basal lamina *in vivo*. In their second paper,[3] α-dystroglycan is proposed as a Schwann cell receptor for the *M. leprae*–laminin complex and these primary rat Schwann cell cultures lacked the integrin $\beta 4$ subunit. Authors suggest that the presence of both $\alpha 2$-laminin and α-dystroglycan is responsible for restricting leprosy infection to the peripheral nervous system as these molecules are absent in the central nervous system.

Adherence of *M. leprae* to host cells has been well studied. Complement-mediated adherence to macrophages is known to occur via mannose receptors and not via FC receptor.[4] The work of Lad and Mahadevan[5] suggests involvement of the carbohydrate moiety. There are indications that adherence of *M. leprae* to Schwann cells is mediated through a lipid/polysaccharide moiety, i.e. antigens such as LAM and PGL 6,7 and to a 25–28 kDa glycoprotein obtained from peripheral nerve homogenates of humans and rats.[8] The fibronectin, an ECM protein, and a component of basal lamina, is also shown to be involved in *M. leprae* binding to Schwann cells.[9] Therefore a multitude of factors have been shown to play a role.

Further, in their hypothesis, Freedman and coworkers describe bacillation of 'myelinated fibre' Schwann cells which in turn leads to demyelination and associated sensory impairment observed in leprosy. *In vitro* studies by Rambukkana and coworkers,[2] i.e. binding assays using immobilized ECM proteins, primary rat Schwann cell cultures, Schwann cell neuron co-cultures and using cryosections of wild type and dystrophic dy/dy mice forms the basis of this hypothesis. In the second paper, Rambukkana and co-workers[3] have also used immortalized human Schwann cells; these, however, showed decreased binding to *M. leprae* as compared to primary rat Schwann cells.

We believe that the following points have direct relevance to the molecular mechanisms of *M. leprae* infection and nerve damage in leprosy. First and foremost, it has been well established that in human leprosy *M. leprae* are rarely seen in the Schwann cells of the myelinated fibres (only

* Correspondence: V. P. Shetty

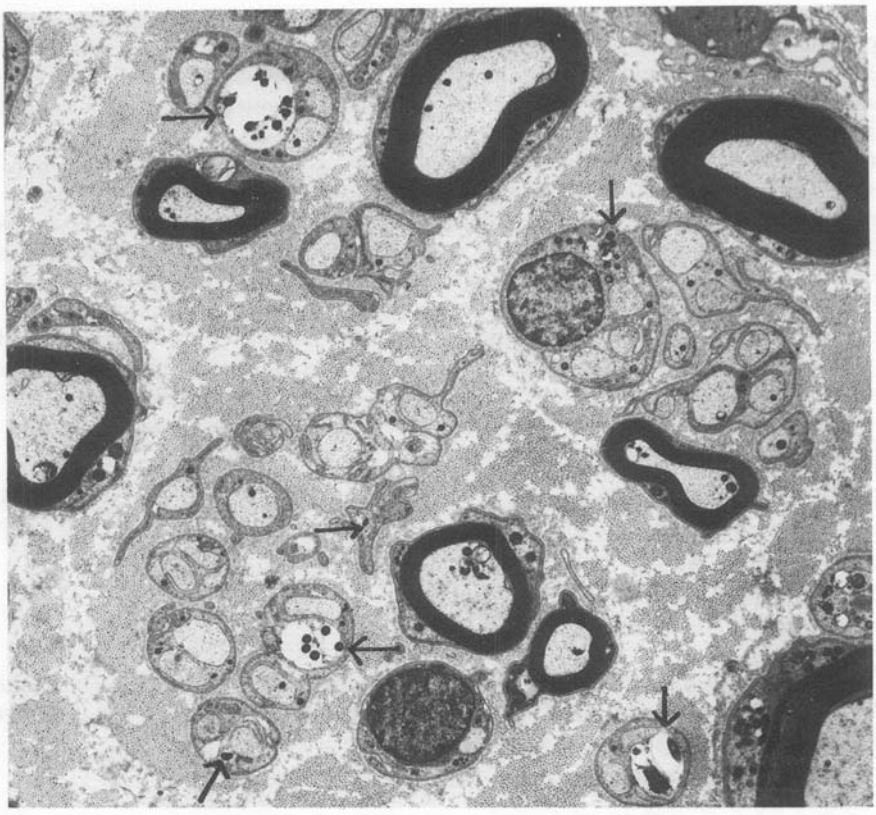

Figure 1. Sural nerve biopsy obtained from an untreated lepromatous leprosy case at an early stage of infection. Note the presence of large globi of solidly stained bacilli in six of the nonmyelinated fibre schwann cell units (arrow) (magnification × 3500).

4–5% in advanced lepromatous leprosy). The Schwann cells of the non-myelinated 'C' fibres are the ones to harbour bacilli (70–80%) (see Figure 1) in the early stage[10,11] This implies that there is no direct correlation between demyelination and infection with *M. leprae* in the Schwann cells. Secondly, and more importantly, this difference also needs to be explained in extrapolating the results of Rambukkana and co-workers that was using culture system.

Since the lamina forms the component of the basal lamina in both non-myelinated and myelinated fibre Schwann cells; and if the spread of *M. leprae* into the nerve is via the circulation as has been proposed by Freedman *et al.*, the preferential bacillation of the non-myelinated fibre Schwann cells remain unexplained.

Secondly, it is also important to note that in almost all the animal models studied so far *M. leprae* rarely enters the peripheral nerves and in particular the Schwann cells; this is not so in humans. The mouse foot pad model for leprosy is extensively investigated from the point of view of understanding peripheral neuropathy associated with leprosy. Reproducible pathological changes in the sciatic nerves following foot pad inoculation with *M. leprae* are well documented.[12,13] However, *M. leprae* and inflammatory cells are conspicuously absent in these lesions.[13] In armadillos, despite disseminated infection with *M. leprae*,[14] the nerve and the Schwann cells in particular seldom show invasion of *M. leprae*. In an attempt to infect Schwann cells *in vivo* through intraneural inoculation with viable *M. leprae*,[15,16] xenografting of leprous nerve into immunosuppressed mice[17] has been undertaken.

It was concluded that Schwann cells in mice are highly resistant to *M. leprae* infection, regardless of route of infection.

Following foot pad inoculation in mice, *M. leprae* colonizes the striated muscle fibres.[18] In armadillos, predominance of endothelial cell bacillation has been reported.[19] In our view, this difference could be a reflection of a species-specific difference in tissue tropism.

Qualitative and quantitative morphological studies of leprous nerves, on the other hand, have shown that atrophic changes in the axonal compartment precede demyelination, indicating the axon to be the primary site of damage.[11,20,21] In an independent study, Shetty and coworkers[22] report detection of multitude of *M. leprae* antigens (including LAM and PGL) in the axonal compartment of both tuberculoid and lepromatous leprosy nerves. In a recent study by us (unpublished data) using SDS-PAGE and WB analysis, it was noted that inoculation into the hind foot pads of S/W mice, with both viable and heat killed *M. leprae*, brings about loss of immunoreactivity to major cytoskeletal proteins of sciatic nerves, again supporting the role of antigens. The model suggested by Freedman and co-workers may have some relevance in this regard, and *M. leprae* antigens such as LAM/PGL and not integral *M. leprae* binding to myelinated fibre Schwann cells may lead to disruption of the cytoskeletal proteins leading to demyelination.

The Foundation for Medical Research
84-A, R.G. Thadani Marg
Mumbai 400 018, India
Fax: 91-22-4932876 c/o 2662735
(E-mail: frchbom@bom2.vsnl.net.in)

M. P. SAVE
V. P. SHETTY

We are publishing this letter with sincere apologies to the authors. The letter was submitted in reply to the article by Freedman et al. 1999 (How leprosy affects peripheral nerves. *Lepr Rev* **70**: 136–139.) The letter was accepted in August 1999 but was then lost.

References

[1] Weinstein DE, Freedman VH, Kaplan G. Molecular mechanism of nerve infection in leprosy. *TIMS*, 1999; **7:** 185–186.
[2] Rambukkana A, Salzer JL, Yurchenco PD, Tuomanen EI. Neural targeting of M*ycobacterium leprae* mediated by the G domain of the laminin-α2 chain. *Cell*, 1997; **88**: 811–821.
[3] Rambukkana A, Yamada H, Zanazzi G *et al.* Role of α-dystroglycan as a Schwann cell receptor for *Mycobacterium leprae. Science*, 1998; **282**: 2076–2079.
[4] Shlesinger LS, Horwitz M. Phagocytosis of *Mycobacterium leprae* by human monocyte derived macrophages is mediated by complement receptors CR1(CD15), CR3(CD11b/CD18) and INF-γ activation inhibits complement receptor function and phagocytosis of this bacterium. *Immunology*, 1991; **147**: 1983–1994.
[5] Lad SJ, Mahadevan PR. Adherence of *Mycobacterium leprae* to macrophages as an indicator of pathogen induced membrane changes. *Ind J Med Res*, 1982; **76**: 804–813.
[6] Itty BM, Mukherjee R, Antia NH. Adherence of *Mycobacterium leprae* to Schwann cells *in vitro. J Med Microbiol*, 1986; **22**: 277–282.
[7] Choudhury A, Mistry NF, Antia NH. Blocking of *Mycobacterium leprae* adherence to dissociated Schwann cells by antimycobacterial antibodies. *Scand J Immunol*, 1989; **30**: 505–509.
[8] Suneetha L M, Satish PR, Suneetha S *et al. Mycobacterium leprae* binds to a 28–30 kDa phosphorylated glycoprotein of rat peripheral nerve. *Int J Lepr*, 1997; **65**: 352–356.
[9] Schorey JS, Li Q, McCourt DW *et al.* A *Mycobacterium leprae* gene encoding a fibronectin binding protein is used for efficient invasion of epithelial cells and Schwann cells. *Infect Immun*, 1995; **63**: 2652–2657.
[10] Shetty VP, Mehta LN, Irani PF, Antia NH. Study of the evolution of nerve damage in leprosy: Part I - Lesions of the index branch of the radial cutaneous nerve in early leprosy. *Lepr Ind*, 1980; **52**: 5–18.
[11] Shetty VP, Jacobs JM, Antia NH. The pathology of early leprous neuropathy. *J Neurol Sci*, 1988; **88**: 115–131.
[12] Mehta LN, Shetty VP, Antia NH. Study of early nerve lesions in mice infected with *M. leprae. Lepr Ind*,1976; **48**: 31–35.
[13] Shetty VP, Vidyasagar PB, Antia NH. Study of the evolution of nerve damage in leprosy. Part III—sciatic nerve lesion in mice inoculated with *M. leprae* with nerve conduction velocity correlates. *Lepr Ind*, 1980; **52**: 26–47.

[14] Job CK, Sanchez RM, Hastings RC. Manifestations of experimental leprosy in the armadillo. *Am J Trop Med Hyg*, 1985; **34**: 151–161.
[15] Shetty VP. Animal model to study the mechanism of nerve damage in leprosy: a preliminary report. *Int J Lepr*, 1993; **61**: 70–75.
[16] Shetty VP, Antia NH. Animal model for leprous neuropathy: our contribution. *Ind J Lepr*, 1996; **68**: 95–104.
[17] Shetty VP, George V. Xenograft studies in leprous neuropathy. *Int J Lepr*, **54**: 133–135.
[18] Weddell AGM, Palmer E, Rees RJW. The fate of *M. leprae* in CBA mice. *J Pathol*, 1971; **104**: 77–92.
[19] Scollard DM, McCormick G, Allen JL. Localization of *Mycobacterium leprae* to endothelial cells of epineurial and perineurial blood vessels and lymphatics. *Am J Pathol*, 1999; **154**: 1611–1620.
[20] Jacobs JM, Shetty VP, Antia NH. Teased fibre studies in leprous neuropathy. *J Neurol Sci*, 1987; **79**: 301–313.
[21] Jacobs JM, Shetty VP, Antia NH. A morphological study of nerve biopsies from cases of multibacillary leprosy given multidrug therapy. *Acta Neuropathol*, 1993; **85**: 533–541.
[22] Shetty VP, Uplekar MW, Antia NH. Immunohistochemical localisation of mycobacterial antigens within the peripheral nerves of treated leprosy patients and their significance to nerve damage in leprosy. *Acta Neuropathol*, 1994; **88**: 300–306.

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Letter to the Editor

GUIDE TO ELIMINATING LEPROSY AS A PUBLIC HEALTH PROBLEM

Editor,

The World Health Organization has recently published a 'Guide to Eliminate Leprosy as a Public Health Problem' (1st edition, 2000. World Health Organization, WHO/CDS/CPE/CEE/2000.14, 40 pp.). This Guide was distributed to the participants to the meeting on Intensification of Elimination Activities in the African Region, held in Maputo last September. The objective is that this Guide will be widely distributed, after local adaptation, in the most leprosy endemic countries.

This guide is beautifully presented, with very clear pictures, and can certainly contribute to disseminate knowledge about leprosy and basic ways to tackle it. In that sense, it incontestably fulfils a need, and we can only applaud the initiative.

I am afraid, however, that it is too much simplified in some aspects, and can thus be a source of errors or of services below minimal acceptable standards. My main concerns are related to the following aspects.

With regard to signs of leprosy, it is said that 'a leprosy patient is someone who has a skin patch or patches with a definite loss of sensation, and has not completed a full course of treatment with multidrug therapy'. Anaesthetic patches are of course important; but if leprosy is only suspected in case of anaesthetic patches, then the most infectious cases will not be diagnosed and transmission will not be reduced. It is well mentioned that 'other signs of leprosy include reddish or skin coloured nodules or smooth, shiny diffuse thickening of the skin without a loss of sensation', but if it is not said that in these cases it is necessary to refer the patient to confirm the diagnosis, possibly through slit-skin smear examination, it could lead to gross over-diagnosis. Certainly, the availability of a high quality laboratory for smear examination is not a prerequisite for introducing MDT services, but discarding smear examination altogether is going too far away in that direction.

It should be made clear when to refer difficult cases, not only for diagnosis, but also in case of complications.

Up till now, the general rule was that the monthly dose intake should be supervised at the health centre. Some exceptions were possible for patients living far away, or during the rainy season, or in other special conditions. For these patients, several months of treatment could be given at a time. After that, a reliable person from the patient's neighbourhood would be made responsible for the supervision of treatment. Now, there is a strategy called 'accompanied MDT' which, I fear, is going too far. If all newly detected patients are simply asked whether they would prefer to collect their treatment from the health centre at regular intervals or to take all the blister packs with them, it is to be expected that most patients would prefer to receive the whole treatment at once; it looks so much easier. It is even possible that health staff will favour that course of action, as it discharges them from their responsibility and means much less work for them. Treatment will not be supervised at all. So, there is no chance to know whether patients still take their drugs or not, and in which way. Will they be sufficiently convinced, after a single visit to the health centre, of the importance of treating themselves regularly for 1 year? If they stop treatment, the health staff will have no way of knowing. Will patients have clearly understood what is expected from them, what are the possible complications and what they are supposed to do in case of new symptoms? No further contact with the patient during the treatment period means a reduced chance

to detect reactional episodes, or to emphasize messages or education on how to avoid new disabilities.

There is no mention of peripheral nerve examination, or of VMT/ST.

The target audience of this booklet should be clearly stated.

I hope that these remarks can help the National Leprosy Control Programme Managers in deciding what adaptations they will bring to this Guide before it is widely distributed in their country.

ETIENNE DECLERCQ

Damien Foundation
Boulevard Leopold II, 263
1081 Brussels
Belgium
(e-mail: edeclercq@damien-foundation.be)

Teaching Materials and Services

Schieffelin Leprosy Research and Training Centre: Course schedule for 2001

Course	Qualifications	Duration	Commencing date	Fees		
				India	SAARC	Others
I COURSES MORE THAN 1 YEAR DURATION				Rs.	US$.	US$
General						
1) Laboratory Technicians	+2 passed, Science graduates preferred	12 months	Jul. 02–Jun. 30	10,000	675	750
1) Diploma in Prosthetic & Orthotic Engineering	+2 passed, Graduates preferred (with science subjects)	30 months	Jul. 02–Jun. 30	15,000	750	1,500
3) Medical Records Technologist	+2 passed	15 months	Jul. 02–Sep. 30	5,000	250	600
Related to Leprosy						
4) Physiotherapy Technicians'	+2 or P.U.C. Passed. (with science subjects)	9 months	Jul. 02–Mar. 30	5,000	250	700
II COURSES LESS THAN 1 YEAR DURATION						
General						
1) Course of Medical Education	(for 4 modules) (for 1 module)	8 weeks	(on request)	15,000 4,000	400 100	750 150
2) Health Education		8 weeks	(on request)	5,000	200	400
Related to leprosy						
1) Basics of Leprosy	Medical personnel engaged in leprosy work	1 week	Jan. 22–Jan. 27 Jul. 23–Jul. 28	1,500	50	100

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2)	Medical Aspects of Leprosy	Medical personnel engaged in leprosy work	1 week	Jan. 29–Feb. 03 Jul. 30–Aug. 04	2,000	65	150
3)	Surgical Aspects of Leprosy	"	1 week	Feb. 05–Feb. 10 Aug. 06–Aug. 11	2,000	65	150
4)	Eye in Leprosy	"	1 week	Feb. 12–Feb. 17 Aug. 13–Aug. 18	1,5000	50	100
5)	Laboratory aspects in Leprosy	"	1 week	Feb. 19–Feb. 24 Aug. 20–Aug. 25	2,000	50	100
6)	Epidemiology & Control Rehabilitation/POD	"	1 week	Feb. 26–Mar. 03 Aug. 27–Sep. 01	2,500	70	150
Note: 1–6 can be taken all together or any specific module also can be taken. If all the modules are taken, the rates are					10,000	300	700
7)	Non-Medical Supervisors visors'	Qualified paramedical workers with a minimum of 5 years experience in the field	2 months	Apr. 02–May. 31	5,000	300	600
8)	Smear Technicians'	+2 passed (with science subjects)	3 months	Feb. 05–May. 05 Sep. 02–Dec. 01	2,000	100	350
9)	Para-Medical Workers'	+2 passed, graduates preferred	4 months	Jul. 02–Oct. 31	5,000	300	600
10)	Shoe-Makers'	V standard with knowledge of English preferred	6 months	Jan. 01–Jun. 30 Jul. 02–Dec. 31	800	55	200
11)	Eye care in Leprosy	Non-medical personnel	1 week	Sep. 03–Sep. 08	1,000	70	200
III COURSES AFFILIATED TO OTHER COLLEGES							
1)	Basics of Physiotherapy in leprosy	Undergraduates in BPT	1 week	By arrangement	1,000	35	
2)	Basics of Occupational therapy in Leprosy	Undergraduates in occupational therapy	1 week	By arrangement	1,000	35	
3)	Nursing				50/day		
4)	Internship for Physiotherapists and Occupational Therapists	Undergraduates in PT and OT	–	By arrangement			

IV IN-SERVICE TRAINING							
1)	In-service training in Medicine, Surgery, surgical rehabilitation, Pathology, Laboratory Technology, Ophthalmology & Epidemiology and Leprosy Control	For qualified medical personnel/health professionals		By arrangement	250	10 (per week)	25
				For other amenities	50	5 (per week)	10
2)	Medical Record Keepers	+2 passed with proficiency in typing and good English	2 months	By arrangement	2,000	100	
3)	Refresher Course in Skin Smears	Trained laboratory technicians	2 weeks		1,000	70	200

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Non A/c Double occupancy	= Rs. 150/- per day
Non A/c extra bed	= Rs. 50/- per day
A/c Single occupancy	= Rs. 200/- per day
A/c Double occupancy	= Rs. 300/- per day
A/c extra bed	= Rs. 100/- per day
Food: Indian Diet: Vegetarian	= Rs. 120/- per day/Non-vegetarian = Rs. 200/- per day
Western Diet	= Rs. 240/- or US $ 6

How to Reach Karigiri: Madras is connected to all the major cities of India by Air. From Madras Airport the fare for taxi is approximately Rs. 1000/- route -> Ranipet - Tiruvalam - Sevoor - Karigiri Hospital. There are also many buses which operate between 05.00 hrs and 22.00 hrs. from Madras to Vellore. From Vellore take any Taxi or Auto which costs Rs. 150 and Rs. 100 respectively or else you can take a prepaid taxi or electric train to the City Railway station (Central station), about 20 kms away from airport. From there take any train to Katpadi Railway station (13 km. away from Karigiri). From Katpadi to Karigiri on Auto will cost Rs 80/-. If you want to be met at Katpadi or at Madras Airport, please let us know well in advance.

Contact/Mailing Address:

The Training Director, Training Department
Schieffelin Leprosy Research & Training Centre
Karigiri 632 106, Vellore District
Tamil Nadu, SOUTH INDIA.

Tel: +91/41674227, +91/41674229, +91/41674221 (Director) +91/41674215 (Training Director)
Fax: +91/41632103, +91/41671274
E-mail: slrtckrg@md3.vsnl.net.in

Publications on tuberculosis

The following (all available free of charge) have recently been brought to our attention:

From the Office of Publications, WHO, 1211 Geneva 27, Switzerland

1 TB. A Clinical Manual for South-East Asia. WHO/TB96.200 (SEA).

Spiral-bound, A5 format, 143 pages, adapted for South-East Asia from 'TB/HIV: A Clinical Manual' by Mukund Uplekar, previously Foundation for Research in Community Health, Bombay, India. 1997.

2 Treatment of Tuberculosis. Reinforced paperback, 77 pages, intended as guidelines for national programmes. WHO/TB/97.220. 1997.

3 Guidelines for the Management of Drug-resistant Tuberculosis. Reinforced paperback of 47 pages. WHO/TB/96.210 (Rev.1). 1997.

4 Guidelines for the Control of Tuberculosis in Prisons. WHO with International Committee of the Red Cross. WHO/TB/98.250. 1998.

5. What is DOTS? A guide to understanding the WHO-recommended TB control strategy known as DOTS. WHO/CDS/CPC/TB/99.270. 1999.

From the International Union against Tuberculosis and Lung Disease (IUATLD), 68, Boulevard Saint-Michel 75006, Paris, France

1. The Public Health Service National Tuberculosis Reference Laboratory and the National Laboratory Network. Minimum Requirements, Role and Operation in a Low-income Country. 110 pages, plus appendices.
2. Technical Guide. Sputum Examination for Tuberculosis by Direct Microscopy in Low Income Countries. Fifth edition, 2000. Strongly bound paperback of 25 papers. Main headings include: Sputum microscopy; the sputum smear microscopy laboratory; the staining method; microscopic examination of sputum smears; recording and reporting smear microscopy results; quality assurance; disinfection, sterilization and disposal of contaminated materials; biosafety in the microscopy laboratory and materials management. Translation into French is under discussion. Copies of the previous Fourth edition are still available in Spanish. (As with the other items above, the fifth edition is available free in reasonable numbers to *bona fide* applicants. It deserves wide distribution to schools of medicine, nursing, laboratory technology, para-medical and front line health workers.)

Topics in International Health CD-ROM series

A new CD-ROM series is ideal for use in teaching or for students to use on their own for self-directed learning.

It was developed by the Wellcome Trust for use as an interactive educational resource for medical and life science students, researchers and healthcare professionals.

Each colourful CD-ROM is composed of three complementary resources:

- A selection of interactive tutorials providing many hours of learning
- An excellent searchable collection of hundreds of high quality images
- A glossary of medical terminology

Each title focuses on a disease or group of diseases of worldwide importance. The CD-ROM series was commended in the 1999 BMA Medical Books Competition, Electronic Media category.

Titles available:

- NEW Acute Respiratory infection (due for publication Spring 2001)
- Diarrhoeal Diseases
- HIV/AIDS
- Leishmaniasis
- Leprosy

- NEW Malaria – 2nd Edition
- Nutrition
- Schistosomiasis
- Sexually Transmitted Diseases
- Sickle Cell Disease
- Trachoma
- Tuberculosis

Further information: http://www.cabi.org/catalog/journals

The Wellcome Trust, London: training fellowships in infectious diseases for scientists from tropical and developing countries

The Trust recognizes the continuing threat of infectious diseases in tropical and developing countries and wishes to develop research expertise that is sustainable in these countries, in order to address health problems arising from infectious diseases. The awards are intended to provide both training and research experience for applicants, who must be based in a developing or tropical country. The training will be obtained at international centres of excellence in either the UK, Republic of Ireland, or any country in the developing or restructuring world, and a substantial period of research will be undertaken in the applicant's home country. For instance, a 4-year award would normally include a minimum of 2 years' research in the home country over the period of the award. AIDS/HIV-related studies relevant to tropical regions are fully supportable.

Eligibility

Applications are invited from postdoctoral basic scientists or postdoctoral medical graduates of up to 6 years' research experience who are nationals of developing countries. Applications may be considered in exceptional circumstances from those who are educated to first-degree or Master's level, who are able to demonstrate substantive potential for research and operational leadership and who have research experience equivalent to a PhD, a evidenced by their publication record. Applicants will wish to become independent research scientists through high-quality research into infectious diseases of regional significance to their home country.

The research proposal must include a clear argument outlining the relevance of the project to the home country. Applications will be assessed on the basis of the candidates' s achievements in research, the scientific merit of the proposal and the appropriateness of the research for the proposed location. The nature of the training component and the training site chosen must be appropriate for the proposed research.

Funding

Awards will be for a maximum of four years and will be non-renewable. Fellowship support may include a salary or stipend appropriate to the countries in which the candidate will be studying or working, as well as project-dedicated and travel expenses. All expenses must be fully justified. Consideration may also be given to the expense of attending a course leading to a recognized qualification in a discipline relevant to the fellowship research programme.

Application procedure

The preliminary approach to the Trust should include an outline of the proposed research, an approximate budget and curriculum vitae of the applicant. This should be accompanied by a written

guarantee of subsequent employment from the host institution in the applicant's home country and a letter of support from a suitable sponsor at the training institution. Applications will be considered throughout the year.

Further information about the initiative can be obtained from:

The Grants Section (Tropical)
The Wellcome Trust
London NW1 2BE, UK
Tel: +44 (0)20 7611 8409
Fax: + (0)20 7611 7288

Information is also available on the Wellcome Trust website: www.wellcome.ac.uk
NB Applicants may not apply for more than one Trust fellowship scheme at any one time.

The Wellcome Trust, London: Health Services Research Awards for Medicine in Developing Countries

The Trust has a long-standing interest in tropical medicine research, and awards are offered to encourage research into the effectiveness of health interventions in developing countries. Applications for these awards may relate to any infectious or noninfectious disease that is of importance in tropical regions. Cancer research and HIV-related studies relevant to tropical regions are also acceptable.

Health services research is defined as the identification and quantification of healthcare needs and the quantitative study of the provision and use of health services to meet them. Such research is usually multidisciplinary and should preferably involve formal links to ministries of health or non-governmental organizations, and provide evidence that the research findings may be adopted in policy and practice.

Project grants in tropical health services research

These awards will provide research costs for up to 3 years for studies that focus upon issues relating to the effectiveness of health services in tropical countries. Applicants for project grants must hold an established post in a eligible university or research institute in the UK or Republic of Ireland, or in a developing country.

Project grant proposals in tropical health services will be considered three times a year. Applications will be considered by the next available meeting of the advisory committee.

The Trust offers a range of awards for UK and overseas nationals who wish to undertake research in any branch of the natural or clinical sciences that has a bearing upon human or animal health. Further schemes that may be relevant to individuals with an interest in tropical medicine are available. Details of all awards are available upon request from the Trust or can be found at www.wellcome.ac.uk

Enquiries should be directed to:

The Grants Section (Tropical)
The Wellcome Trust
183 Euston Road
London NW1 2BE, UK
Tel: +44 (0)20 7611 8641
Fax: +44 (0)20 7611 7288
E-mail: tropical@wellcome.ac.uk

Free International Newsletters and Recommended Journals on Subscription

The fourth edition of *Free International Newsletters and Recommended Journals on Subscription* gives details of 145 newsletters, magazines and journals relating to health and disability issues which are available free or at low cost to readers in developing countries. Also listed are 22 newsletters and journals available on subscription.

This resource list has been used as a valuable networking tool to learn about the activities of other organizations working regionally and internationally. It also provides a rich source of core materials to build up resource centres and provide up-to-date health information at little extra cost.

The titles appear in alphabetical order and are indexed by subject, geographic focus and language. A brief description of each publication is given and the full address of each publisher, so that publications can be ordered directly. As more and more journals and newsletters are available electronically via the Internet or e-mail, the web address and e-mail address of electronic titles are also provided.

Healthlink Worldwide is seeking to make this publication widely available to individuals and organizations involved in health and disability issues and training. Single copies are available free to readers in developing countries. For others the cost is £5/$10 and includes postage. Bulk copies are also available free to organizations that are able to distribute the publication through their own channels. To obtain further information, or to place an order, please contact: Toyin Ikotun, Editorial Assistant, Information Production & Management Team. e-mail: ikotun.t@healthlink.org.uk, telephone (direct): +44 20 7539 1578.

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News and Notes

New NGO to promote elimination of leprosy

A new NGO by the name Leprosy Elimination Alliance (LEA) had been launched in Chennai (India) recently. The main objectives of the organization, set up in August 2000, with a special focus on India, are (a) to promote and advocate the cause of leprosy and leprosy elimination, (b) to promote exchange of information and ideas on leprosy elimination among leprosy workers and others, (c) to monitor progress towards leprosy elimination and assist towards development of better strategies and methods to achieve the goal in collaboration with other interested parties and (d) to produce and distribute among leprosy workers and others publications on leprosy elimination.

As part of its objectives LEA expects to produce and distribute a quarterly publication by the name *Bulletin of Leprosy Elimination Alliance* by the beginning of the year 2001. The publication will be available free of cost to all those interested in leprosy elimination. The chairman of Leprosy Elimination Alliance is Dr S. K. Noordeen, formerly Director of the Action Programme for Elimination of Leprosy at the World Health Organization headquarters in Geneva.

Further details on the NGO and its publications can be obtained from:

Dr. S. K. Noordeen, Chairman, Leprosy Elimination Alliance, Flat 1-A, K. G. Valencia, 57, 1st Main Road, Gandhinagar, Chennai - 600 020, India. Phone: (044) 4456337, Fax: (044) 4456338, Email: noordeen@eth.net

Report on the First Meeting of the WHO Technical Advisory Group on Elimination of Leprosy. WHO/CDS/CPE/CEE/2000.4. Geneva 2 and 3 May 2000

The 36-page Report should be studied in the original by all concerned with the elimination of leprosy.

The *Introduction* reads as follows:

Today, leprosy is no longer the dreaded disease that it used to be, and leprosy patients face a far better future than ever before. Over the last 15 years, there have been significant advances in reducing leprosy prevalence, thereby reducing the grossly disfiguring consequences of the disease, its pain and suffering, and the social stigma it causes. However, this does not mean that all the problems in leprosy have been resolved, nor does it mean that we can afford to slacken our efforts towards the elimination of the disease as a public health problem. In spite of the fact that the profile of the disease is much milder, and that disability among new patients is quite low, the social image of leprosy has not greatly changed in many parts of the world; this is all too well reflected in the attitude of the community, particularly towards individuals disabled or disfigured due to the disease.

The tremendous progress made in conquering leprosy in recent years has been largely due to the widespread implementation of multidrug therapy (MDT), which cured leprosy patients of their disease, and significantly reduced its burden in leprosy-endemic countries. This progress is essentially the result of a World Health Assembly resolution in 1991, which committed all leprosy-endemic countries of WHO to a global target of reducing the prevalence of leprosy to less than one case per 10,000 population, and setting the end of the year 2000 as the target date to achieve this.

These targets were extremely useful in generating political commitment to push ahead and achieve the results, which would otherwise not have been possible. This is well demonstrated by the fact that, since 1985, the prevalence of leprosy has been reduced globally by nearly 85% and nearly 10 million leprosy patients had been cured. A large part of the credit for this should go to the determination and commitment of leprosy-endemic countries in their elimination efforts, the consistent efficacy of MDT in curing leprosy, and the all-round support provided by various partner agencies of WHO, including international donor non-governmental organizations (NGOs).

The epidemiological situation in leprosy was also very favourable in many countries, especially in Africa. The progress made so far is more than just in numbers and statistics alone. The contribution of the progress in relation to reduced physical, psychological and social suffering, as well as an improved health image for countries is truly immeasurable.

Despite the fact that several major national programmes will not reach elimination by the set target date of 2000, WHO is confident that with extra efforts, the goal will be within reach of all the remaining countries by the end of 2005. But there must be no complacency in the years ahead, as many of these countries still face formidable problems that will require new and innovative solutions based more on local realities than before. And there are even more areas within countries where, long after the country has attained elimination at the national level, continuing and sustained efforts will still be required to reach similar targets at both the provincial and district levels.

In a major effort to achieve elimination in all countries by 2005, the *Global Alliance for the Elimination of Leprosy (GAEL)* was launched in Abidjan, on 15 November 1999. In addition to WHO, the core members of the Alliance are the governments of the 10–12 most endemic countries, the Nippon Foundation, the International Federation of Anti-Leprosy Associations (ILEP), and Novartis. The Alliance will cooperate closely with the World Bank, the Danish International Development Agency (DANIDA) and other non-governmental organizations. The objective of the Alliance is to exchange information so that better services may be delivered to the underserved communities. WHO will provide secretariat services and technical leadership, and the Government of India has agreed to chair the first year of the Alliance.

In order to advise WHO on effective implementation of the intensified strategy and the monitoring of its progress, particularly in the areas of capacity building, MDT supply, communication and information, and monitoring and surveillance, the Director-General decided to establish a *Technical Advisory Group on Elimination of Leprosy (TAG)*. The Group consists of six independent experts selected for their expertise in leprosy and programme management with particular reference to public health, epidemiology, community mobilization and advocacy, operational research, and disability prevention. The group forms a strong team with balanced technical expertise and geographical representation.

The terms of reference of the Group are:

- to review and monitor implementation of the intensified strategy for elimination of leprosy;
- to advise WHO on new strategies and approaches if necessary;
- to review progress towards elimination;
- to give technical advice and guidance on efforts towards elimination of leprosy;
- to identify gaps and obstacles that may deter smooth operations and find solutions in order to facilitate implementation of planned activities in the field;
- to address research issues.

The first meeting of the *WHO Technical Advisory Group on Elimination of Leprosy* was held in Geneva on 2 and 3 May 2000.

And the *Executive Summary*:

Leprosy elimination stands at a critical and extremely difficult juncture. This is partly because the commitment to eliminate leprosy in many endemic countries is beginning to slacken (among decision-makers and in the field). Moreover, those areas that are easy to reach and to work in, have been

effectively covered. The residual problem is far more difficult – from all perspectives – and is further complicated by structural inadequacies in local health services. Even today, people in many areas do not have ready access to diagnosis and MDT (including those with long-standing disease). Therefore, achievements will no longer be sustainable if significant numbers of hidden cases remain undetected and accessibility to treatment services remains difficult.

There is a need to critically review existing strategies and to develop pragmatic approaches adapted to field realities in order to facilitate the delivery of essential activities leading to elimination of leprosy at the local level. Failure to do so could be misinterpreted as a failure of the current elimination strategy as well as the technology behind it. This working paper outlines the progress made towards the elimination of leprosy as a public health problem, the challenges to be faced and the opportunities we have to accelerate activities at the most peripheral level.

Combinatorial chemistry; a revolution in drug discovery, but still in need of logic to drive it forward

A leading article in the *British Medical Journal*, volume 321, 9 September 2000, pages 581–582, reviews the present situation with regard to combinatorial chemistry, the process by which millions of molecular constructions can be created and tested simultaneously and which now underlies the speed of new drug development. Extracts of the text include the following:

A key component of any drug discovery programme is synthetic organic chemistry, which has analogies with Lego blocks. Chemical building blocks are assembled according to precise rules, creating molecules of increasing complexity. Some of these blocks hold others in position, while others are capable of interacting with a biochemical target (such as the active site of an enzyme or a receptor). Some blocks are there to modify the metabolism of molecules and others modify the overall structure to improve drug delivery. 'Combinatorial chemistry' is the process by which millions of molecular constructions can be created and tested simultaneously and which underlies the speed of new drug development.

BMS-201038 is Bristol-Myers Squibb's first molecule to get through to the clinic whose origins were within a focused combinatorial library of chemicals synthesized by robotics. Other examples have come from Merck, which has described several combinatorial cases, such as a series of molecules that interact with somatostatin receptors.

Medicinal chemists traditionally synthesized a handful of different molecules, submitted them for test in appropriate biological assays, and then waited for the results. As the results came through, the chemists would modify the design of the molecules and a new generation would be created and retested. Although modern refinements, such as drug design aided by computer, became an integral part of the process, this archaic regimen consistently represented one of the rate limiting steps in getting drugs from discovery to market.

Recent advances, however, have significantly compressed the discovery component of the pharmaceutical timescale for research and development. The study of gene sequences (genomics) has improved the identification of useful points of therapeutic intervention; combinatorial chemistry has generated massive numbers of molecules for testing; and screening using high throughput techniques has automated the process of doing large numbers of biological assays. All have reduced the discovery-to-market time from the conventional 10–14 years to the 5–8 years claimed today.

Not surprisingly, in today's entrepreneurial world many new companies are capitalizing on combinatorial chemistry. The combination of performing organic synthesis on solid support, along with automation and robotics, allows a single chemist to synthesize thousands of different molecules in 1 week, which can then be evaluated for biological activity in a high throughput manner. Such collections of molecules are referred to as libraries. Most of the pharmaceutical and biotechnology industries are now carrying out some form of combinatorial chemistry in their laboratories. Those who recognized the merits of this science early on have now got molecules in the clinic that came from

combinatorial chemistry and high throughput screening. The promise of considerably shortened discovery-to-market time has become a reality.

As chemistry becomes capable of being able to synthesize the universe of possible organic structural permutations, and high throughput screening is approaching the capacity to screen everything against every biochemical target ever identified, we wonder if science is taking a backward step towards empiricism. We will have a huge barrage of information without having thought about how all the pieces fit together. Today's approach is in danger of becoming haphazard, with little logic driving it forward.

The costs associated with this 'big, dumb science' approach are not trivial. But as new information is analysed and a more focused rationale emerges, the costs should diminish. People who begin to understand the processes involved, rather than simply generating large quantities of data, are going to rediscover the pleasure of becoming scientists again.

A clinical prediction rule for nerve function impairment in leprosy patients

The above is the title of an important paper by Richard P. Croft, formerly working in the The Danish-Bangladesh Leprosy Mission (DBLM), Nilphamari, Bangladesh, published in the *Lancet*, volume 355, May 6, 2000, pages 1603–1606. The Summary reads as follows:

Background: Nerve-function impairment (NFI) commonly occurs during or after chemotherapy in leprosy and is the key pathological process leading to disability and handicap. We describe the development of a simple prediction rule for estimating the risk of NFI occurrence.

Methods: New leprosy cases who presented to a centre in Bangladesh were recruited and followed up for 2 years in a field setting. We used multivariable regression analysis by Cox's proportional hazards model to identify predictive variables for NFI. Discriminative ability was measured by a concordance statistic. Internal validity was assessed with bootstrap resampling techniques.

Findings: 2510 patients were followed up for 2 years, 166 developed NFI. A simple model was developed with leprosy group (either paucibacillary leprosy [PB] or multibacillary leprosy [MB]) and the presence of any nerve-function loss at registration as predictive variables. Patients with PB leprosy and no nerve-function loss had a 1.3% (95% CI 0.8–1.8%) risk of developing NFI within 2 years of registration; patients with PB leprosy and nerve-function loss, or patients with MB leprosy and no nerve-function loss had a 16.0% (12–20%) risk; and patients with MB leprosy with nerve-function loss had a 65% (56–73%) risk.

Interpretation: Our prediction rule can be used to plan surveillance of new leprosy patients. Patients at low risk of NFI may need no follow-up beyond their course of chemotherapy (6 months); patients with intermediate risk need a minimum of 1 year of surveillance; and patients with high risk should have at least 2 years of surveillance for new NFI. Current recommendations for surveillance of patients with leprosy (for the duration of chemotherapy only) exclude an important group of patients who are at risk of developing NFI after completion of treatment.

The importance of these observations is referred to in another, recent publication in the *Lancet*: 'Strengths and weaknesses of leprosy elimination campaigns', Volume 355, June 17, 2000, page 2089, by Pieter Feenstra, Department of Health, Royal Tropical Institute, Amsterdam 1092, AD, Netherlands. He proposes that the incorporation of the measures described by Croft *et al.* would improve the efficiency of disability prevention in Leprosy Elimination Campaigns.

Remote access: tracking TB, leprosy and HIV in northern Malawi

The Wellcome Trust 'Karonga Prevention Study', based in Chilumba in the Karonga District of Malawi, is the fortunate inheritor of vital data and facilities from a major study of leprosy begun by LEPRA (the British Leprosy Relief Association) more than 20 years ago. The legacy of the LEPRA work included an

equipped and staffed field centre, a unique set of interviews and medical examinations on more than 250 000 people, and almost 100 000 stored blood samples. 'Initially, LEPRA funded us to set up a population laboratory to study the natural history of leprosy, employing tools developed during the 1970s,' explains Professor Paul Fine of the London School of Hygiene and Tropical Medicine. 'But as time went on we got hooked on the broader issues of mycobacterial immunity and the relationships between leprosy, TB and HIV.'

Karonga District was originally chosen as a research site because of its high incidence of leprosy. Beginning in 1979, a team of 75 locally recruited field workers surveyed the entire district, painstakingly conducting house-to-house surveys, and interviewing and examining 112 000 people. By 1984, the programme had developed into the largest and most detailed study of leprosy every carried out, with descriptions of the pattern of leprosy according to age, sex, area, socioeconomic status, household contacts, family histories, immunological status, and whether participants had received a Bacille Calmette–Guérin (BCG) vaccine. This vaccine is a live bacterium related to *Mycobacterium tuberculosis*, the cause of TB, and (in theory) stimulates immunity against mycobacterial pathogens.

One of the surprise findings of the study was that BCG vaccination provided good protection against the leprosy-causing bacterium (*M. leprae*), but not against *M. tuberculosis*—in stark contrast to northern Europe, where BCG successfully induces immunity to *M. tuberculosis*. The team went on to undertake the biggest vaccine trial ever carried out in Africa. This study of 120 000 people showed that a BCG vaccine that protects 70% of UK children offered no protection against the lung form of TB. The work showed conclusively that the UK vaccine simply does not work in Malawi, and ruled out the suggestion that differences in efficacy could be due to the use of different vaccines in the two countries.

'What seems most likely,' says Professor Fine, 'is that the high frequency of other mycobacterial infections in tropical countries impairs the effect of BCG vaccines. A person's immune response to other mycobacteria—which are found in the soil and even tap water—may offer some 'natural' protection against *M. tuberculosis*, but it also blocks or masks the effect of the vaccine.'

By the 1990s leprosy was on the decline in Africa. 'When we first went out to Karonga there were ten times as many leprosy patients as TB patients, but by 1995 the situation had reversed,' says Professor Fine. In part this was due to the success of their leprosy control programme, but it also reflected the rapid spread of HIV-infected individuals more susceptible to TB infection.

With LEPRA shifting its focus to India, where most of the world's leprosy cases are now found, Professor Fine and his team were loth to close a site they had invested so much effort in setting up, particularly with so much valuable data on the population collected and so many intriguing puzzles yet to be solved. Why did the BCG vaccine protect against leprosy but not TB in that population? Why was HIV having a greater effect on TB than leprosy? What factors predisposed individuals to infection and to disease? The desire to answer such questions led Professor Fine to apply for Wellcome Trust funding.

From the LEPRA work, records and pedigrees exist for more than 250 000 people, with details on whether they have suffered from leprosy or TB. One aim of the Trust-funded study is to use these data to identify genes that predispose to leprosy or TB. Key to this aim is the coding system used in the LEPRA projects, which uniquely identifies each participant and enables complex family trees to be constructed. Making use of these data, field workers are now going back to selected individuals to collect blood samples. This material is being sent to the Wellcome Trust Centre for Human Genetics in Oxford, where Professor Adrian Hill's group is looking at the frequency of candidate genes—previously identified genes that might potentially affect susceptibility to leprosy or TB—in patients and uninfected individuals living in similar conditions.

In the second part of the genetics project, family trees are allowing field workers to identify affected sibling pairs and to undertake a 'fishing' exercise for susceptibility genes. If a particular genetic marker turns up in affected sib pairs more often than would be expected by chance, then that marker is probably associated with the disease—generally, it will be close to a susceptibility gene.

These studies could have significant medical benefits: 'If we could identify the genetic factors which control the immune response this would have major implications for diagnosis, vaccination and treatment of TB,' says Professor Fine.

In the immunology arm of the study, researchers are exploring how the BCG vaccine affects the way people respond to different mycobacteria. Blood was collected from 635 Malawian teenagers who had never been exposed to BCG. The blood samples were exposed to 35 different mycobacterial antigens. Then two-thirds of the individuals were vaccinated, and the same tests were repeated 1 year later. 'We will be able to see how people respond to a large panel of different mycobacterial antigens, and interpret patterns as reflecting differences in background exposure and genetics,' says Professor Fine, who in this area is collaborating with Professor Jenefer Blackwell of the Wellcome Trust Centre for Molecular Medicine in Cambridge. Results will be compared with those from an identical study being carried out by Professor Fine and his colleague Hazel Dockrell, among school children in Essex (funded by the World Health Organization and LEPRA), a comparison that will help to explain why the vaccine protects against TB in the UK but not in rural Africa.

The group is also looking at patterns of TB, and at the evolution and interaction of the dual epidemics of TB and HIV. With the permission of the Malawian Government, 60 000 blood samples collected since 1980 have been tested for HIV. 'We have been able to identify a few hundred HIV-positive patients from the early phase of the epidemic there, and are comparing their families with controls to see the effect of the epidemic on morbidity, mortality, demography and social structure over the course of 20 years,' says Professor Fine. 'Ultimately, such information could contribute towards control of the disease.'

The project is one of many remote projects the Trust has funded. Based seven hours' hard drive from the capital, there have been particular challenges in transporting scientific equipment, such as freezers, over dirt tracks to their specially built labs. Another challenge has been recruitment of scientific staff from the West. 'To live in a place like Chilumba you need staff with a pioneering spirit who find self-sufficiency rewarding. This means that you narrow the field in terms of applicants.' Last year, for example, it was all hands to the deck to construct a ford when the bridge to the headquarters was washed away in floods.

The success of the project depends on teams of locally recruited field workers, who travel to remote areas in the Karonga district to collect samples and interview the population. Part of the Trust grant has been used to provide them with 30 Yamaha 125 motorcycles, as well as modern lightweight camping equipment. Although the teams are serviced by support Land Rovers and a camp attendant, they need to be able to carry everything on the back of a motor bike when they go off into the hills.

One aspect of the work that Professor Fine has found particularly rewarding is that the research has made a valuable contribution to the district's health services. 'The population know that in the course of our research we diagnose and care for the tuberculosis, leprosy and skin disease patients in the district. In the early days, the need to examine people for leprosy led to our setting up a general dermatology service. Our staff travel and are known throughout Karonga District, and because of the benefits of the research the people have responded well to us over the years, accepting our intrusive efforts to understand the diseases of this population.'

(Taken from *Wellcome News*, issue 22Q1, 2000)

RELEASE: Leprosy Control Programme in western and mid-western regions of Nepal

We are grateful to the Programme Manager for sending a copy of the report on activities throughout 1999. Four elements are included - leprosy control and prevention of impairment and disability; rehabilitation services to people with disability from causes other than leprosy; addictive drug rehabilitation and support for victims of AIDS. The report includes detailed information on the epidemiological situation and continuing progress, in collaboration with His Majesty's Government in Nepal, towards the goal of elimination. The closing paragraph of the *Preface*, however, adds a word of warning about the additional work and effort still needed to achieve this goal, hopefully by the year 2005:

In the first week of February 1999 the whole of RELEASE worked together to support the National Leprosy Elimination Campaign (NLEC). Starting on World Leprosy Day, the campaign worked in 27 districts of the country, moving from house to house to find backlog cases of leprosy. In just 1 week, we detected 11,961 new cases of leprosy in the country, out of which 1284 were in the Western Region. This doubled the number of registered cases. The cases found included large numbers with extensive disease (MB), pre-existing deformity and children. In view of this, we are reluctant to say that we can achieve leprosy elimination by the end of 2000 AD. The World Health Organisation have already announced an extension to the elimination target, up to 2005 AD, in 12 highly prevalent countries of the world, including Nepal. We believe, however, that with the co-operation of the Government of Nepal, the NGO sector and the active participation of the people of Nepal we can achieve the elimination target before that date.

The THELEP controlled clinical trials in lepromatous leprosy. TDR/IDE/THELEP/99.1

This document, A4 format, 158 pages, edited by Professor Louis Levy, gives a detailed description of a remarkable series of trials from 1977 onwards set up to address two major concerns at that time, namely microbial persistence and drug-resistance in the chemotherapy of leprosy.

The preface by Dr S. K. Noordeen, Director (1994–1998), Action Programme for the Elimination of Leprosy, reads as follows:

During the last quarter century, leprosy work throughout the world has greatly benefited from the fruits of research in several areas including chemotherapy. In this regard, major contributions to research into the chemotherapy of leprosy have come from the Scientific Working Group on Chemotherapy of Leprosy (THELEP), now replaced by a Steering Committee on Chemotherapy of Mycobacterial Diseases (THEMYC), under the UNDP/World Bank/WHO Special Programme on Research and Training in Tropical Diseases, which has worked in close collaboration with the Leprosy Programme of the World Health Organization (WHO). THELEP made important contributions to the development of modern multidrug therapy (MDT) in leprosy, which is currently central to WHO's goal of elimination of leprosy as a public health problem. THELEP/THEMYC's contributions include mapping the problem of dapsone resistance, better understanding of the effectiveness of currently available drugs and their contributions, and development of newer regimens to treat leprosy, including the recently recommended treatment of single-lesion paucibacillary leprosy by administration of a single dose of the combination of rifampicin, ofloxacin and minocycline. Apart from these contributions, the THELEP/THEMYC clinical and field trials have engendered a whole new outlook in the chemotherapy of leprosy and built research capacities in many leprosy endemic countries.

This compilation of studies carried out under THELEP brings out the tremendous collaborative efforts made by scientists all over the world to improve the treatment of leprosy as well as to understand better some of the basic issues in the chemotherapy of leprosy.

I am confident that the compilation of this work will be of considerable use to researchers and leprosy workers alike.

THE ORGANIZATION OF THE THELEP TRIALS

The THELEP controlled clinical trials of combined chemotherapy of lepromatous leprosy were designed as a multi-centre trial, the first two[1] participating centres being the Institut Marchoux,

[1]Initially, no decison was taken to limit the participation to two centres. However, it soon became clear that the laboratory at the NIMR, at that time the only laboratory capable of work with *M. leprae*-infected TR mice, could cope only with the specimens to be supplied by the first two centres, and that no additional trials could be undertaken among patients with lepromatous leprosy until additional laboratory facilities had been developed. In fact, the THELEP SC considered the development of such additional facilities a matter of high priority.

Bamako, Mali, and the Central Leprosy Training and Research Institute, Chingleput, South India. Among the requirements of such a trial were; i) a Standard Protocol; ii) standard forms for reporting the clinical and laboratory data; iii) a central facility for data-storage and retrieval; and iv) a coordinator, responsible for overseeing the work of the treatment centres and collaborating laboratories. Finally, it was agreed that the data resulting from the trials were to be the joint property of those participating in the trials – the collaborating laboratories and treatment centres, together with the THELEP Steering Committee (SC), and a 'Subcommittee on Clinical Trials of the Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases', composed of the participants, was informally established. The scientific papers resulting from the trials were to be published under the rubric of the Subcommittee.

As was noted in the preceding chapter, preparation of the *Standard Protocol for Chemotherapy Trials in Lepromatous Leprosy* was commissioned by the Planning Committee, and the Standard Protocol was reviewed and adopted by the SWG at its first meeting, and subsequently published in both English and French. Standard data forms were then prepared with the assistance of the WHO Health Statistical Methodology Unit (HSM), as it was then known, and also published in both English and French.

Consequent to decisions taken by the SC, the clinical laboratory studies incident to the trials were carried out at the two centres, histopathologic examinations were performed by Dr A. C. McDougall at the Slade Hospital, Oxford, normal mice were inoculated in Professor G. R. F. Hilson's laboratory at St George's Hospital Medical School, for the purpose of measuring susceptibility of the patient-strains of *M. leprae* to dapsone, and TR mice were inoculated in the Laboratory for Leprosy and Mycobacterial Research, National Institute for Medical Research (NIMR), for the purpose of detecting persisting *M. leprae*. Dr M. F. R. Waters was appointed Clinical Trials Coordinator, assisted in Bamako by Professor S. R. Pattyn. Dr Waters also organised and conducted a Standardisation Workshop, to ensure that uniform criteria and procedures were employed at the two treatment centres. Finally, Mr J. L. Duppenthaler, HSM, was responsible for data-storage, retrieval and analysis.

DISCUSSION AND CONCLUSIONS

At the time that the THELEP programme began, the two major concerns of the scientists working in the area of chemotherapy were the phenomena of microbial persistence and drug-resistance. It appeared certain that relapse caused by the emergence of drug-resistant *Mycobacterium leprae* could be prevented by employing combined (multidrug) therapy, particularly combinations including rifampicin, which was known to exert a powerfully bacterial action against the organism. It was also clear that control of leprosy by chemotherapy would be possible only if chemotherapy of finite duration were curative; long experience with dapsone as monotherapy had demonstrated that neither patients nor the treatment services could be expected to comply with treatment of indefinitely long duration. However, rifampicin as monotherapy had recently been shown to be incapable of eradicating *M. leprae*. And it was feared that persistence of viable organisms in the lepromatous patient, whose immune response to the organism was known to be deficient, would lead inevitably to relapse after cessation of the chemotherapy. Workers hoped that a multidrug regimen could be discovered that was curative, i.e. capable of eradicating persisting *M. leprae*, thereby preventing relapse.

As its first priority therefore, the THELEP Scientific Working Group undertook to conduct comparative trials of multidrug regiments, employing measurements of the proportion of persisting *M. leprae* as the index of efficacy. Trials of regimens of varying intensity, all including rifampicin, were mounted in Bamako, Mali, and in Chingleput, South India, involving finally a total of 215 patients with multibacillary leprosy, who were believed to have had no previous treatment. Persisting *M. leprae* were detected in 43 (7.8%) skin-biopsy specimens among a total of 554 specimens obtained at intervals of 3, 12 and 24 months from 38 of a total of 203 patients during treatment with five combined drug regimens. The proportion of specimens in which persisting organisms were discovered could not be shown to vary with regimen or duration of treatment. The regimen consisting of a single large initial dose of rifampicin plus daily dapsone was not shown to be less effective, in terms of the proportion of specimens in which

persisters were detected, than regimens consisting of rifampicin, dapsone and clofazimine or protionamide, each drug administered daily. The average patient's burden of persisting *M. leprae* was calculated to lie in the range 50,000–250,000 at each of the intervals, numbers of organisms much smaller than those that had been anticipated. These data were consistent with information regarding the relatively small risk of relapse after cessation of chemotherapy among patients with multibacillary leprosy, information that was not available when the clinical trials were mounted. In addition, the small numbers of persisting *M. leprae*, which appear to reflect the role of rifampicin as a component of the drug-combination, provided strong support to the multidrug regimen recommended for treatment of multibacillary leprosy by the World Health Organization Study Group on Chemotherapy of Leprosy for Control Programmes.

An important by-product of these trials were the data on primary resistance to dapsone. Approximately 37% of the 131 patients with lepromatous leprosy admitted into the THELEP controlled clinical trials in Bamako and Chingleput, whose *M. leprae* obtained from pretreatment biopsy-specimens could be tested in mice, were found to harbour dapsone-resistant organisms, and were presumed to represent instances of primary resistance to dapsone. Although the majority of these patients harboured strains of a low degree of resistance, 20% harboured organisms of an intermediate degree of resistance. These data represented an important addition to the published evidence, obtained from surveys among similar numbers of patients, of the increasing frequency with which primary resistance to dapsone was being encountered, and served to emphasize the need to employ multidrug therapy in the treatment of patients with multibacillary leprosy.

Fourteen cases of jaundice were observed among 212 patients – 9 among the 51 patients who had been treated by the maximal regimens, that included daily administration of rifampicin for the entire 2 years of the trials, and 5 among the remaining 161 patients. The probability that jaundice could have occurred with such different frequencies among two samples drawn from the same population of patients is only 0·001, i.e. jaundice was significantly more frequent among those patients treated by rifampicin administered daily for 2 years. These results suggest that daily administration of rifampicin as a component of multidrug therapy, as has been advocated in the USA and in other countries, in which the added cost of daily, compared to monthly, administration of the drug is not important, carries an increased risk of hepatotoxicity.

In summary, the THELEP controlled clinical trials of combined chemotherapy of previously untreated lepromatous leprosy at Bamako and Chingleput demonstrated that, after treatment by one of several combined-drug regimens, all of which included rifampicin, persisting *M. leprae* comprised only a tiny proportion of the total population of organisms harboured by the average patient. In addition, these trials confirmed the alarmingly large proportion of such patients who present with their *M. leprae* already resistant to dapsone. And, finally, daily administration of rifampicin, as opposed to its monthly administration, could not be shown to be more efficacious therapeutically, whereas daily administration appeared to carry a greater hazard of hepatotoxicity.

After the THELEP controlled clinical trials had been mounted, but well before their completion, it became apparent that persisting *M. leprae* do not pose as great a threat of relapse to the patient with multibacillary leprosy, once chemotherapy has been completed, as was earlier believed. As a consequence, the importance of these trials was diminished even before the results had been assembled. Nevertheless, the results of the THELEP trials have great relevance to the current efforts to control leprosy, and lend strong support to the intermittent, rifampicin-containing regimen recommended by the WHO Study Group for the treatment of multibacillary leprosy.

Twenty countries pledge to wipe out TB

Ministers from the 20 countries most burdened by tuberculosis marked world tuberculosis day last week by agreeing a framework for action aimed at 'consigning to the history books' a disease that currently claims 2 million lives a year.

The Amsterdam Declaration was signed at a conference convened by the World Health Organization (WHO) and the World Bank, partners in the new Stop Tuberculosis initiative. The initiative aims to increase coverage of the highly effective directly observed treatment, short course (DOTS) programmes from 25% of patients with tuberculosis to 70% in 5 years.

The countries, representing 80% of the 8 million new cases of tuberculosis each year, committed themselves to supporting a global partnership agreement. This will help to finance and support national tuberculosis programmes, especially in countries worst hit but least able to afford to take action. The WHO and the World Bank are to work with governments to implement the agreement, which should begin in the autumn.

The initiative also plans a global fund for tuberculosis and a global drug facility to seek extra financing and ensure the supply, distribution, and monitoring of tuberculosis drugs. The conference heard that 'substantial and sustained funding' was needed. The health minister for Thailand, Korn Dabbaransi, hoped that other countries 'would be convinced of our true need for financial and manpower resources.'

The economic and development importance of investment was emphasized with figures showing that 95% of the 20 million people with tuberculosis lived in the developing world. Three quarters of cases in those areas, moreover, were in the most economically productive age group of 15 to 54 year olds.

Dr Arata Kochi, director of the initiative, said that more funding was being sought.

Source: *British Medical Journal*, volume 320, 1 April 2000

Persistence of *Mycobacterium tuberculosis* in macrophages requires enzyme essential for metabolism of fatty acids

Writing from the Howard Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, New York and other centres in the USA, J. D. McKinney and colleagues have recently submitted a letter to *Nature*, volume 406, 17 August, 2000, pages 735–738 entitled 'Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glycoxalate shunt enzyme isocitrate lysase'. The first paragraph (summary) reads as follows:

Mycobacterium tuberculosis claims more human lives each year than any other bacterial pathogen. Infection is maintained in spite of acquired immunity and resists eradication by antimicrobials. Despite an urgent need for new therapies targeting persistent bacteria, our knowledge of bacterial metabolism throughout the course of infection remains rudimentary. Here we report that persistence of *M. tuberculosis* in mice is facilitated by isocitrate lyase (ICL), an enzyme essential for the metabolism of fatty acids. Disruption of the *icl* gene attenuated bacterial persistence and virulence in immune-competent mice without affecting bacterial growth during the acute phase of infection. A link between the requirement for ICL and the immune status of the host was established by the restored virulence of Δicl bacteria in interferon-γ knockout mice. This link was apparent at the level of the infected macrophage: activation of infected macrophages increased expression of ICL, and the Δicl mutant was markedly attenuated for survival in activated but not resting macrophages. These data suggest that the metabolism of *M. tuberculosis in vivo* is profoundly influenced by the host response to infection, an observation with important implications for the treatment of chronic tuberculosis.

and the last paragraph of the letter:

Persistence of bacteria and chronicity of infection are hallmarks of tuberculosis. Patients with chronic tuberculosis are thought to harbour bacteria in various metabolic states, ranging from active cell growth and division to stationary phase. Conventional drugs target processes required for bacterial cell growth and division, such as cell-wall biogenesis and chromosome replication. Poor activity against slow- or non-growing bacteria is thought to be an important reason why conventional drugs take so long

to eradicate infection. Therefore, our demonstration that ICL promotes persistence of infection by enhancing bacterial survival within inflammatory macrophages makes it an attractive new target for chemotherapy. The development of ICL inhibitors will be facilitated by the recent solution of the three-dimensional structure of *M. tuberculosis* ICL in association with the prototypic inhibitors 3-bromopyruvate and 3-nitropropionate. Future efforts will focus on the development of ICL inhibitors as new drug candidates with preferential activity against persistent bacteria.

TB transplant to TDR: rapid intake, minimal rejection

A recent issue of *TDR News* (UNDP/World Bank/WHO special Programme for Research & Training in Tropical Diseases) No 62, June 2000, carries the following news about TB:

Tuberculosis is slotting rapidly into the TDR portfolio and 'culture'. In February 2000, a scientific working group on TB met to make recommendations for research and capability strengthening activities in TDR. It clarified TDR's role and niche among the ongoing alliances and initiatives in TB. Later in the month, the recommendations were presented to the TDR Scientific and Technical Advisory Committee (STAC). Altogether, TDR input to TB research is now looking quite distinct, with significant funds already identified to support some of the proposed work. Further interest in TB will definitely have been stimulated by the Ministerial Conference on TB and Sustainable Development held in Amsterdam, 22–24 March 2000, from where the final declaration called for acceleration of both basic and operational research.

The scientific working group (SWG) recommended that TDR adopt a two-pronged policy on TB research:

- health systems and services research (HSSR) – being the most neglected area in TB research.
- research and development (R&D) of new diagnostics, drugs and vaccines – being an area where TDR has considerable comparative advantage.

The HSSR agenda should be driven by the needs arising from TB control programmes, and the SWG recommended that TDR develop a conceptual framework for the necessary research/control link. TB-HSSR also needs to be established in high burden countries (especially the 22 countries that account for 80% of the world's TB burden) e.g. through building up national TB research institutions, expanding assistance for protocol development, and linking with other TDR diseases.

In R&D of new tools for TB, the first priority is diagnostics. Particularly needed are a replacement for the sputum smear test, a rapid test for rifampicin sensitivity, and evaluations of marketed products, as well as expansion of the specimen bank established by the TB diagnostics initiative and already transferred to TDR. Second, equal priority is to both drugs and vaccines. In drugs, the particular need is to evaluate available antibiotics and 'off the shelf' drugs, and to encourage production by small pharmaceutical companies in the South. TDR will work with other actors in this area – the IFPMA/WHO Roundtable, Stop TB, and the Global Alliance for TB Drug Development. As a partner in the Global Alliance, TDR is delighted to announce the award (on March 24) of US$25 million to the Alliance by the Bill and Melinda Gates Foundation. With respect to vaccines, product profiles, animal models and correlates of protection are specifically needed, and the SWG encouraged TDR to look for ways to speed up vaccine development.

Research capability strengthening activities should be aimed at supporting research throughout these two main areas (HSSR and R&D of new tools). In particular, there is a need to build capacity for conducting field trials of new diagnostics, drugs and vaccines, and for carrying out post-regulatory assessments and functional genomics research.

TB was welcomed into the TDR disease portfolio by the Joint Coordinate Board (TDR's top governing body) in 1999, which requested STAC to prepare plans of action and a focus for TB research, assuming that a budget of US$5.5 million for 2000–2001 could be raised. Already some of these funds

are available, from, amongst others, the Rockefeller Foundation, Sweden (SIDA/SAREC), the Swiss Development Agency, Stop TB and WHO.

(*TDR News* is published three times a year Fax (+41) 22 791 4854.
E-mail: tdrnews@who.int, Web site: http://www.who.int/tdr)

Reforms to the health sector must retain vertical programmes like those for tuberculosis

The following letter recently appeared in the *British Medical Journal*, volume 320, 24 June 2000, from Sir John Crofton, emeritus professor of respiratory diseases, University of Edinburgh, United Kingdom:

EDITOR—Health sector reform has become the policy urged on poor countries in the developing world. Basically it entails transferring responsibility for health services and health budgets to local communities. I am sympathetic to this approach. But its uncritical application by governments has a dangerous obverse.

Vertical programmes—for instance, central coordination and monitoring of the World Health Organization's DOTS (directly observed treatment short course) programme for control of tuberculosis—may be discouraged. The programme may be suddenly abolished. The economy of scale resulting from national bulk buying of antituberculous drugs disappears. The tuberculosis experts in the Ministry of Health, who provide leadership and coordination and who monitor the programme, are dispersed to other jobs. Suddenly there are no drugs for tuberculosis, either centrally or at the periphery, and no control programme.

I am told that this has already occurred in Zambia and Ethiopia. It almost occurred in Bangladesh. It is threatening to occur in many other countries.

With HIV infection and multidrug resistance, the World Health Organization has declared tuberculosis to be a global emergency. It is a desperate race against time to establish good national tuberculosis control programmes, especially in the 22 countries that contain four fifths of the world's cases. National control programmes would prevent the development of multidrug resistance—always the result of bad doctoring—before the alliance of multidrug resistance with HIV infection creates an almost untreatable pandemic (tuberculosis is no respecter of frontiers).

It is essential to retain the economies of scale offered by the central purchase of drugs and basic diagnostic equipment. It is essential to retain control of central monitoring and coordination and gradually to hand over the major responsibility of the service to local communities as their skill develops. Just as in community development projects in the United Kingdom, professionals continue to be needed in the background to pick up the bits when a local administration fails.

When I raised this problem at a recent symposium on global health the representative of Save the Children supported me. He said that the child immunization programme in Uganda had almost collapsed for the same reasons. I have just visited the School of Tropical Medicine in Liverpool and had discussions with people working on tropical disease problems in poor countries. Although sympathetic with the concept of health service reform, many are disturbed by the possibility of the sudden abolition of vertical programmes with no real provision for their effective replacement.

Prospects for global tuberculosis control under the DOTS strategy. WHO/TB 98.251

This document of 34 pages, A4 format, is by Christopher Dye and colleagues, Global Tuberculosis Programme, World Health Organisation, 1211 Geneva 27, Switzerland November 1998. It describes the development of an age-structured mathematical model to explore the principles of tuberculosis control

under DOTS (Directly-Observed Therapy, using a Short-course regimen), and to forecast the impact of improved case finding and cure on TB epidemics in different regions of the world.

The closing page of the *Discussion* (pages 15 and 16) reads as follows:

There are numerous uncertainties in making projections with mathematical models, and their effects are only partly reflected in the bounds on our estimates. Most of what we know about the natural history of tuberculosis – which determines model structure and parameter values – comes from studies in industrialized countries, and yet we are most interested here in the prospects for TB control in the developing world. Apart from the ranges attached to model parameter values, there are critical but unpredictable external variables. We do not know precisely how many TB cases arise each year, and how many are currently found and cured. Nor can we be sure of the course of HIV epidemics, which particularly affect projections for Africa and Asia. However, the principles of TB control revealed by our analysis do not-depend on the exact results of model calculations. And, whilst predictions of the *numbers* of cases and deaths between now and 2020 are subject to great uncertainty, we can be more confident (roughly to the extent indicated by lower and upper bounds) about comparisons of the preventable *fraction* of the TB burden when control targets are met by different dates.

Even if WHO targets are met by year 2010, three-quarters of the global TB burden would *not* be averted over the next 23 years. Better diagnostics, drugs and vaccines, plus targeted preventive therapy, would undoubtedly help. But new control measures with the potential to have a major impact may not be available for years. Meanwhile, the most pressing tasks are to find ways of achieving higher cure rates, and reaching more cases, in the principal endemic countries of the world.

HIV: Durban (South Africa) conference warns HIV levels on the rise again

With the subheading 'No reprieve on AIDS, experts warns west', the *Guardian* newspaper (UK) carried the following in its issue of July 12, 2000:

Western complacency over AIDS is ill judged because globalization means we cannot escape the consequences of major epidemics in Africa or Asia, a British expert warned in Durban yesterday.

Roy Anderson of the UNAids collaborating centre for epidemiological research, based at Oxford University, said the numbers of people infected with HIV were rising again.

'We have not by any means seen the worst of this problem' he said. 'It will get progressively worse over the next 10 to 15 years. People often cite the 1918 influenza pandemic, which caused over 30m deaths. This is going to be many factors bigger than that.'

There is an assumption among some in the west that the problem of Aids has gone away, he said. Drugs are suppressing the virus levels in infected people so that they can live normal lives.

But researchers in the field are finding that, as people live longer, so the pool of people infected with HIV who could potentially pass it to others increases, and there is no way of eradicating the virus.

In the US now, mortality is beginning to rise again very slowly said Professor Anderson. 'The virus becomes resistant to the drugs if people do not take their medication exactly as stipulated by doctors. There has also been an increase in risk-taking behaviour by people who assume the drugs will protect them from AIDS and an early death.'

In the UK, there has been a very slow but progressive spread among heterosexuals.

'It is creeping along. We're at the beginning of an epidemic for heterosexuals,' said Prof Anderson, warning that the full enormity of the global disaster is difficult to estimate. 'People don't fully understand that the timescale is 40 to 50 years for this epidemic.'

The crisis in sub-Saharan Africa, where 25m people are infected with HIV, is a tragedy for the world, he said.

'This is the biggest infectious disease problem that has faced humanity in the course of human history. We can't isolate ourselves... What happens in Africa does matter to us. There are also economic

factors too. If labour markets are not there because of the enormous burden of disease, that will have a knock-on effect.'

Anthony Fauci of the National Institute of Allergy and Infectious Diseases in the US yesterday told the AIDS 2000 conference in Durban that even when virus levels had been undetectable in infected people for considerable periods, they soared again within a month of stopping drug treatment.

The drugs appear to have little effect on a reservoir of virus in body tissues, even when they suppress it in the blood, he said.

But vaccines and cheaper drugs are, nevertheless, the hope for the future. Dr Fauci said he was running trials on interrupted drug regimes - where people whose HIV is under control are taken off medication every other week. His preliminary results show that the viral levels did not go up during the week off.

He warned that nobody should experiment alone with stopping their medication, because of the dangers of the virus developing resistance to the drugs.

'The numbers are still small and we need to look at the critical effect after a year or two. But it is encouraging,' he said.

Links

www.unicef.org.uk/breakthesilence
Campaign focusing on AIDS orphans
www.unaids.org
Joint UN programme on HIV/AIDS

News from the Damien Institute

Damien News, Summer/Monsoon 2000, carries the news that Father William F. Petrie will be leaving India in the near future, after 25 years of work on behalf of leprosy patients, mainly in Orissa. He is handing over the Directorship of the Damien Institute to Brother James Rukavina who will be based at N/5-525 Nayapalli, Bhubaneswar 751 015, Orissa, India. *Damien News* draws attention to the remarkable support given by Father Bill and his group over a long period of years, including surgical operations for disabled patients, free medical check-ups, training of para-medical workers, provision of tricycles, wheel-chairs and other aids, planting of fruit trees and the building of over a thousand houses for leprosy patients families. Father Bill will now spend some time on the island of Molokai, where Father Damien lived, worked and died. We wish Brother James and the Damien Institute every success in their future work in this high-endemic part of India.

Th1 and Th2 cytokine responses

The *British Medical Journal*, volume 321, 12 August 2000 has an interesting commentary on these responses. The first three paragraphs read as follows:

Cytokines are the hormonal messengers responsible for most of the biological effects in the immune system, such as cell mediated immunity and allergic type responses. Although they are numerous, cytokines can be functionally divided into two groups; those that are proinflammatory and those that are essentially anti-inflammatory but that promote allergic responses.

T lymphocytes are a major source of cytokines. These cells bear antigen specific receptors on their cell surface to allow recognition of foreign pathogens. They can also recognize normal tissue during episodes of autoimmune diseases. There are two main subsets of T lymphocytes, distinguished by the presence of cell surface molecules known as CD4 and CD8. T lymphocytes expressing CD4 are also

known as helper T cells, and these are regarded as being the most prolific cytokine producers. This subset can be further sub-divided into Th1 and Th2, and the cytokines they produce are known as Th1-type cytokines and Th2-type cytokines.

Th1-type cytokines tend to produce the proinflammatory responses responsible for killing intracellular parasites and for perpetuating autoimmune responses. Interferon gamma is the main Th1 cytokine. Excessive proinflammatory responses can lead to uncontrolled tissue damage, so there needs to be a mechanism to counteract this. The Th2-type cytokines include interleukins 4, 5, and 13, which are associated with the promotion of IgE, and eosinophilic responses in atopy, and also interleukin-10, which has more of an anti-inflammatory response. In excess, Th2 responses will counteract the Th1 mediated microbicidal action. The optimal scenario would therefore seem to be that humans should produce a well balanced Th1 and Th2 response, suited to the immune challenge.

International charity offers health and loans

The following is extracted from the *British Medical Journal*, volume 321, 9 September, 2000, page 592:

An international charity that provides small loans for women in impoverished communities had taken the unusual step of providing a health education service alongside its credit facilities. And from an early evaluation, it seems that financial and physical health messages mix well together.

The effectiveness of the village health bank scheme, run by Project Hope, whose international headquarters is in Millwood, Virginia, has been evaluated by researchers from the George Washington University Center for International Health, in Washington, DC.

The evaluation found that the health status of members of the village status of members of the village health banks (as measured by six critical health indicators including contraceptive use, annual cancer screening, postnatal care, immunization, child growth, and prevalence of diarrhoea) was 14% higher than that of participants receiving credit-only services and not health education.

Now Project Hope wants to expand its village health banking scheme, from the countries where it operates at present (Honduras, Ecuador, Malawi, Guatemala, and Peru), to several other developing countries, including Thailand and the Dominican Republic.

The idea of village health banking is simple. Women in the most impoverished areas of the world are given the opportunity to start their own businesses by being offered small loans of about $80 (£53). The businesses usually centre on buying and selling food and local textiles to rural areas.

Groups of about 25 women form one bank. They elect a committee to manage the bank and take responsibility for recouping the loans. Women are charged commercial rates of interest on their loans as experience shows that these repayments can be and are achieved.

Interleaved into regular health bank meetings is the health promotion part of Project Hope's scheme.

One member of each bank is elected as the health officer and provided with health education materials that are geared to the problems of the local population. 'The two elements of the scheme–credit and health education–are introduced in an integrated fashion. We appreciate that the best way to deliver health education is by an integral process so we have made provision for that by allowing a local health officer to facilitate a learning opportunity with the bank members,' explained John Bronson, director of the income generation programmes at Project Hope.

Typical discussions centre on the environment, nutrition, child health, and women's health. Activities that have been successful include cleaning up rubbish, operating health fairs, transporting a nurse to weigh and examine small children, and identifying locally available nutritious food.

With training from Project Hope, the banks quickly become self sufficient. General rules are laid out, and compliance has been excellent. Loans need to be repaid every 4 months, and if they are repaid successfully the member qualifies for a larger loan next time.

Altogether there are 400 banks that have benefited over 20 000 women and their families. Over $7m has been loaned out in five years with less than $20 000 not repaid–a default rate of less than 2%.

Although the project boasts a repayment rate that would make most banks envious, the real rewards of the scheme are the effects on people's lives. In Honduras, for example, women participating in village health banks showed an average improvement of 38% across important health indicators and were rated 37% better than women who did not belong to such banks. With increased wealth these women were able to spend 41% more on basic necessities such as food and medicine.

But is has not all been plain sailing. When some women are excluded from membership of a bank by other women, they can feel resentful. 'We are always advocating having a good mixture of people as members. We constantly argue for a diversity of membership. But the decisions are made by the participants themselves,' said Bronson.

There is a strong wish for the group to succeed, and the group as a whole guarantees the loans, so if a particular woman cannot pay back her part of the loan, the other women have to pay it for her. The result has been that some people with less education or who are less able have found themselves excluded.

'There are also conflicts with spouses and traditional leaders, something that you find with any scheme that empowers women,' Bronson added. 'We have heard of women who have been pulled out of the banks by their husbands.'

On top of these problems, which have occurred everywhere, there have been particular difficulties in operating in Malawi, owing to the exceptional poverty in that country. 'The average income of people in the Latin American countries where we operate is between $30 and $60 a month, but in Malawi, it is $10.

'We have a lot of repayment problems. The challenges are greater there than elsewhere because the people are much worse off. There is a 33% illiteracy rate. On the other hand, we are helping people who really need help, which makes it worthwhile,' Bronson explained.

One of the greatest benefits of running village health banks, is the boost it gives to a woman's self-esteem.

'Village banks can become very powerful groups in their own communities and extend their experience to other community problems,' said Bronson. He cited the example of a bank in Honduras that was wiped out by Hurricane Mitch in late 1998. 'Rather than wait for government or foreign assistance to arrive the people of the bank were proactive. They took charge and were trying to find solutions to their problems so that they could rebuild their lives.'

Further information
www.projhope.org

Liaison Newsletter of the WHO Library and Information Networks for Knowledge

Volume 10, No 3, 1999 of this publication has an Introduction by Irene Bertrand, WHO HQ Library and a series of articles on BIREME, Sao Paulo Brazil, with emphasis on its creation in 1982 of a bibliographical database called LILACS and, more recently of a Virtual Health Library for Latin America and the Caribbean. The Introduction reads as follows:

In our first number of *Liaison* in 1999 we summarized our plans for forthcoming issues, amongst which was to continue our annual geographic theme by featuring Latin America. We are therefore devoting this number to the Latin American and Caribbean Center on Health Sciences Information (BIREME). BIREME was established in 1967 in Sao Paulo, Brazil, as the Regional Medical Library in an agreement between the Pan American Health Organization (PAHO) and the Government of Brazil. It was intended to perform a similar role for Latin America as that taken by the US National Library of Medicine for the USA.

The Regional Medical Library (BIREME) was later named the Centro Latinoamericano y del Caribe de Informacion en Ciencias de la Salud which better reflected its present objectives and functions. In its unique role, BIREME supports the regional network of health science information for the whole Latin

American continent which includes over 600 national and PAHO documentation centres and other specialized networks. It develops and promotes the use of tools for technical databases, e.g. DeCS (Descriptors in Health Sciences) and supports training and research activities in countries for the development of the decentralized LILACS CD-ROM bibliographical database.

BIREME has led the way in health information activities throughout the developing world and its first great achievement was the creation in 1982 of the bibliographical database of regional health literature entitled LILACS (the Latin American and Caribbean Health Sciences Literature database) which indexes some 600 journals as well as books, technical reports, etc. from the region. It aimed to complement the MEDLINE database whose coverage is primarily American and European health journals. Originally in the form of a printed bibliography, LILACS became another 'first' by being issued on CD-ROM in 1989, at approximately the same time as commercial firms were bringing out MEDLINE int he same format. Since then, the original database has been supplemented by others in more specific subject areas such as food legislation, toxicology, etc. LILACS uses the Medical Subject Headings (MeSH) of the US National Library of Medicine to index the local health literature but quickly saw the necessity to complement and expand this thesaurus with its own controlled vocabulary in the area of public health (DeCS – descriptors in health sciences).

The latest major BIREME initiative is the implementation of the Virtual Health Library for Latin America and the Caribbean which integrates its various information sources into a network of products and services on the Internet. It also includes the project SciELO – Scientific Electronic Library – a common model for electronic publishing by the Virtual Health Library.

BIREME is on the leading edge of health information dissemination and must be congratulated on its wide range of innovative activities and the example it sets for all regions to emulate; we thank them for providing the following articles describing their projects, which make up this issue of *Liaison.* In view of its topical importance, the description and development of the Virtual Health Library and its various components takes pride of place. Background information on the LILACS database and the DeCS thesaurus complete the overview of BIREME's remarkable activities.

Further information:

Bireme, Rua Botucatu, 862, 04323-901 - Sao Paulo, Brazil.
e-mail: bireme@bireme.br
http://www.bireme.br.

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Cables: UNISANTE GENEVA
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Telex: 415 416
E-Mail: LIBRARY@WHO.CH

Editors: Mrs I. E. Bertrand and Miss V. L. Paterson

UK Department for International Development

One of DFID's publications, *International Health Matters*, includes the following information:

International Health Matters is a digest of knowledge gained from research funded by the Health and Population Division of the UK Government's Department for International Development (DFID). Each issue reports both completed and ongoing research on a particular subject, indicating implications for policy and practice. *International Health Matters* is published twice a year. A full-text Web version is available at: http : //www.liv.ac.uk/lstm/ihm – cov.html or http : //www.dfid.gov.uk.

Editor: Robert Cole, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA. Tel +44 (0)151 708 9393. Fax: +44 (0)151 707 1702. E-mail: rcole@liv.ac.uk.

Design and desktop publishing: Paula Waugh, Robert Cole.

If you have any enquiries or comments please contact the editor at the above address.

International Health Matters is printed on chlorine-free, environment-friendly paper.

There are a number of ways to access information about DFID's policies and activities:

DFID Internet

As a channel for communicating with external audiences, the Internet is increasingly important to DFID. Through the Internet, 130 million users worldwide can find out about UK development policy and DFID's structure and activities. Information resources available via the Internet include: DFID's Target and Institutional Strategy Papers, *Who We Are* (general information), Advisory Group information (e.g. Health, Education, etc.), Project Evaluations, Publications, Pipeline Projects, Recruitment pages, Research, Press Releases, Speeches.

DFID's Internet address is: http : //www.dfid.gov.uk/.

DFID Public Enquiry Service

DFID operates a public enquiry service which provides information to external callers on the work of the Department. Typical enquiries include requests for information on DFID policies and expenditure, DFID publications, contact details, country programmes and project information. Customers include members of the public, NGOs, businesses, academic, other government departments, other bilateral and multilateral donors.

Tel: 0845 300 4100, (outside UK: +44 1355 84 3132). Fax: +44 (0) 1355 84 3632. E-mail: enquiry@dfid.gov.uk.

DFID's *Centre for Health Information* (CHI) is a useful contact for details about the Department's Health and Population activities. The CHI manages a database providing project-level information from DFID;s *Health Portfolio*, and can provide details of DFID's health activities by country and subject.

Contact: The Centre for Health Information, Health & Population Department, DFID, 94 Victoria Street, London SW1E 5JL. Tel: +44 (0) 207 917 0333. Fax: +44 (0) 207 917 0428. E-mail: CHI@dfid.gov.uk.

New programme explores technologies to challenge disability and improve health care delivery to the poor

Identifying and making better use of appropriate technologies that will improve the lives of poor people

worldwide is the focus of a new £1.2 million Knowledge and Research (KaR) Programme that commenced in December 2000. This innovative programme will encourage the development and use of appropriate disability and healthcare technologies. It is managed by a unique partnership between a management consulting firm, GIC Ltd, and a UK-based health, development and disability communications charity, Healthlink Worldwide. Funding for the programme is being provided by the Department for International Development (DFID).

Three DFID departments are co-operating in the programme, in recognition of the overlapping areas of interest the programme will be covering. The three units are Infrastructure and Urban Development, Social Development and Health and Population. 'This is an exciting opportunity to pool resources and collaborate together to contribute to the global knowledge pool about how to improve the introduction of and use of technologies affecting the lives of poor people', said Director of the KaR Programme, Roger Drew, of Healthlink Worldwide.

The KaR Programme on Disability and Healthcare Technologies will make a total of £1.2 million available over the next 2 years to support a range of projects under the themes of: improving healthcare technologies and infrastructure for poor people and minimizing the detrimental effects of disability on the lives of the poor. The Programme sees technologies as including processes and management practices, organizational and supportive systems and dissemination practices that make them more accessible.

The Programme will support projects that fall within one of three broad indicative categories: development of a new technology, adoption of a newly developed technology and contribution to the wider use of a successful technology. An annual competition will be used to select the projects that will be awarded funding. Full details of the application process are available on the Programme's website (http://www.kar-dht.org). The second round of the competition will begin in September 2001. Projects can be awarded funding for up to 100% of their costs. In selecting projects, the Programme will seek to provide a good balance between small and large projects, between disability and healthcare technologies, among the three categories identified, and ensure broad geographic coverage. 'We're looking for projects that will show how technology can address the priority health problems of the poor and encourage effective health systems', said Programme Manager, Aron Cronin, of GIC Ltd. 'We're also looking for approaches that improve the access of disabled people to their basic rights, and that seek to prevent some of the poverty-based causes of disability'. Communicating the lessons learned and promoting the use of knowledge already generated will be a key part of individual project activity, as well as being a major activity of the Programme itself.

For further information about the Knowledge and Research Programme on Disability and Healthcare Technology, please contact: Roger Drew, KaR Programme Director, Healthlink Worldwide, Tel: +44 20 7539 1577; Fax: +44 20 7539 1580; e-mail: drew.r@healthlink.org.uk, Aron Cronin, KaR Programme Manager, GIC Ltd. Tel: +44 20 7253 7000; Fax: +44 20 7251 3100; e-mail: kar@giclimited.com, Website: http://www.kar-dht.org.

For information on application procedures, please contact Anne-Laure Ropars at GIC by e-mail or fax.

Instructions to Authors

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