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# LEPROSY REVIEW

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**understanding of leprosy and its control**

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## Editor's Choice

This issue of *Leprosy Review* might seem quite slim after the bumper issue that we produced for the Beijing Congress, but it contains what I hope is becoming our regular mix of high quality original papers, useful reviews, teaching articles and comments on topical issues.

The article on *Mycobacterium w* immunotherapy in leprosy looks at the published data on this therapy. Immunotherapy hastens bacterial clearing, but it is not clear whether this translates into significant clinical improvement. Of considerable concern is the observation that there may be an increased risk of reversal reactions in patients given *Mycobacterium w*.

Warwick Britton's comprehensive article on reversal reactions (p. 225) emphasises the importance of nerve damage in reversal reactions and the variable response to steroids. Two papers from collaborations in Nepal follow on from this review. The paper by Weir *et al.* (p. 279) shows that even in careful longitudinal studies of T cell responses to *M leprae* antigens, it is not possible to predict which patients are going to develop a reaction in the next month. The difficulty in detecting and measuring nerve damage is illustrated by the paper of Brandsma *et al.* (p. 257) showing that testers were in good agreement for muscles that were normal or paralysed but not good on an expanded intermediate range. In other words we can't reliably detect subtle losses. However, I suspect that field workers will be relieved to know that they are not going to be asked to measure muscle strength on an 11-point scale.

We continue our theme of women and leprosy with a paper showing that in Chad women do have access to leprosy services for diagnosis and treatment.

At the LEPRO annual general meeting in July we said goodbye and thanked Jenet Batten for all she has done for *Leprosy Review*. She was assistant editor for 17 years and the journal hugely improved and expanded its circulation under her guidance. I and several other editors are very grateful to her for her work and initiative. We wish her well for the future. I would also like to welcome our new assistant editor, Susan Boobis who has a background in pharmacology and publishing. She is also a great enthusiast for electronic media and has an article in this number sampling various medical web sites. I'm sure that under her guidance we are all going to become more web-literate.

DIANA N. J. LOCKWOOD

## Editorial

### MYCOBACTERIUM W IMMUNOTHERAPY IN LEPROSY

Recent trials which demonstrate the benefits of BCG vaccination in preventing all types of leprosy have been followed by trial reports suggesting that immunotherapy may have a role in the treatment of leprosy patients. Extremely thorough work in India identifying, purifying and bringing to trial autoclaved *Mycobacterium w* (M.w.) has been rewarded with very encouraging early trial results from pilot studies<sup>1</sup> and the first relatively small unblinded trials.<sup>2–4</sup> However, there remain a number of important questions to be answered before the role of M.w. can be clearly defined and it can be recommended for routine clinical practice.

WHO multi-drug therapy has proved highly effective in curing bacterial disease, both pauci- and multibacillary cases, with very few relapses. However, treatment of multibacillary cases lasts at least 12 months and complications of reversal reactions, neuritis and erythema nodosum leprosum (ENL) are common, unpleasant, and difficult to treat in many cases. Thus there is most interest in the effects of M.w. on the multibacillary group, for whom a shortening of the duration of treatment, or a reduction in reactions, or a reduction in long-term disability would be very important contributions.

The rationale for immunotherapy with *Mycobacterium w* (M.w.) is that it may boost cell-mediated immunity and therefore lead to increased clearance of bacilli. The published reports comparing vaccinated and unvaccinated individuals seem to confirm that this happens, with Bacterial Index (BI) and viability of *M. leprae* in mouse footpad testing falling more rapidly in BB, BL and LL patients vaccinated with M.w. analysed separately.<sup>5,6</sup> Subgroup analysis should be interpreted cautiously because of small numbers, but the effect is least clear-cut in the LL cases. This is made less easy to interpret because the vaccinated groups started with lower BIs, but is convincing, nevertheless. BCG also seems to cause the same effect<sup>5</sup>. Lepromin conversion rates are higher in those given immunotherapy. These studies of surrogate markers suggest the theoretical basis behind immunotherapy is sound, but does this translate into significant clinical improvement?

The problems with measuring clinical outcome in leprosy are well known, and no scoring system is ideal. Published studies used two outcomes. One was a clinical score (first described by Ramu<sup>7</sup>); the other was release from treatment. Neither is 'objective' (for example fever/no fever or dead/alive); both depend on the assessment of a clinician. It is therefore essential, if they are to be entirely reliable, that there should be double blinding of the highest possible standard. The early studies were unblinded, or semi-blinded; the study reported by Zaheer *et al.* was described as double-blind. This is exceptionally difficult in the case of M.w., as active vaccination causes local induration and erythema, then the formation of a well defined

ulcer, with scar formation from about 3 weeks. Blinding the patient is therefore effectively impossible, and clinicians, who must perform careful examination of the skin both for the scoring system and to decide on release from treatment, will be given a strong hint by the presence or absence of a recent typical scar. Where clinicians believe in a treatment, it is almost impossible not to be influenced by this, albeit unconsciously. The use of plasters on all upper arms to hide scars or lack of scars was used in some trials of BCG where similar problems exist, and those conducting M.w. trials might want to consider this. With this caveat, the first trials are encouraging, with quicker apparent resolution for all multibacillary cases, both in improvement of clinical score and in release from treatment.

The overall bacteriological cure in multibacillary leprosy is unlikely to be significantly improved by immunotherapy, as it is already close to 100% with conventional treatment even in field conditions. It is also unlikely to make any impact on transmission, since cases are non-infectious within 48 h of starting conventional treatment, and almost all transmission occurs before cases are identified or treated at all—hopes that it will ‘provide a big boost in the leprosy eradication campaign’<sup>8</sup>, are therefore misplaced. However, a faster resolution is definitely in the interests of patients, and has clear operational advantages provided it does not carry a heavy price. If immunotherapy does kick-start the cell-mediated immune system to recognize the bacilli and kill them earlier in the disease than conventional treatment, it may hasten bacterial clearance, but at the risk of causing a higher incidence of immune-mediated reactions. These can be very unpleasant and potentially cause life-long disability. Preventing and managing ENL, neuritis and other immune-mediated problems remains one of the great challenges in leprosy, and is often only partially successful. There are theoretical reasons for thinking successful immunotherapy might cause a higher incidence of these problems (and some which might suggest the reverse): the question is—does it? At present, the evidence of this is mixed, but there are serious concerns which will need addressing before immunotherapy can be wholeheartedly recommended.

The early studies were not large enough to detect anything but the grossest evidence of increased reactions, and were not designed to do so. The abstracts of two studies which do address the problem of type 1 reactions give the impression that there was no important difference in rates of reaction between vaccinated and unvaccinated groups, but the data give a less reassuring picture. One study set out to look specifically at this question.<sup>9</sup> This found a 22.6% incidence of reversal reaction in the vaccine group ( $n = 53$ ) and 15% in the control group ( $n = 53$ ), with more severe reactions in the vaccinated group. Only three patients had neuritis—two in the vaccine group, one in the control group. This failed to achieve statistical significance and was reported as showing no evidence of increased neurological damage. However, since there were only 53 patients in each arm, there was little chance of achieving significance, and the failure to prove a difference did not prove that no difference exists.<sup>10</sup> A larger study by the same group was reported by Zaheer *et al.*, with 93 vaccinated patients and 107 controls.<sup>6</sup> This also demonstrated more type 1 reactions, with 35% of vaccinated patients having reactions and only 17% of controls, the difference being most marked in the LL patients. On the other hand, they report fewer episodes of neuritis and ENL in the vaccinated groups, although some of the figures are puzzling, in particular the fact that more LL controls are reported as having neuritis (70) than there are patients in the LL control group (68).

The apparent increase in type 1 reactions and the absence of any long-term follow up data on the very important endpoints of relapse and disability make it inappropriate for immunotherapy with M.w. to be recommended in routine clinical practice at present. Initial

excitement is understandable, but over-hyping M.w. at this stage is unhelpful for patients and those administering programmes on the ground. Properly blinded randomized trials which are large enough to pick up significant differences, particularly in neuritis and long-term disability, will need to be reported before the place of M.w. becomes clear. Further studies are underway in India, and the initial results are sufficiently encouraging that trials in different ethnic groups and settings are warranted at this stage. Meanwhile immunotherapy should only be given in the context of a properly conducted trial. Everybody treating patients with multibacillary leprosy will hope that immunotherapy lives up to its initial promise of faster cure without increased harm, but the case for it is not yet proved.

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REVIEW ARTICLE

## The management of leprosy reversal reactions

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### Introduction

Although *Mycobacterium leprae* infects both skin and peripheral nerves, it is the propensity to nerve damage with resulting disability which marks leprosy as a significant health problem. There have been recent dramatic falls in the prevalence of leprosy following the implementation of multi-drug therapy (MDT) of short fixed duration;<sup>1,2</sup> however, the incidence of new cases has yet to fall significantly in the major endemic countries and the pool of patients with potential nerve damage remains large.<sup>3</sup> Leprosy reactions impact significantly on the management of the disease in two ways. First, the major determinant of nerve damage is inflammation occurring within the perineurium, and this is exacerbated during reactional episodes. Second, the spontaneous and fluctuating course of reactions, often with deterioration occurring after the commencement of MDT, is baffling to the patient and medical worker alike and a significant barrier to compliance. Understanding the mechanisms and natural history of reactions is critical for the early treatment and prevention of nerve damage.

In leprosy, host immunity to the bacillus determines the pattern of the disease,<sup>4</sup> with a spectrum of response. In tuberculoid leprosy (TT), a strong cellular immune response to *M. leprae* limits replication of the organisms, but causes tissue damage to a small number of nerve trunks and skin lesions. The hallmark of lepromatous leprosy (LL) is the absence of T cell recognition of the organism, resulting in uncontrolled proliferation of bacilli, numerous skin lesions and infiltration of dermal nerves and nerve trunks. The bulk of patients have borderline leprosy with retention of some T cell responses and moderate numbers of bacilli and lesions. Although patients with the polar TT and LL forms of the disease are generally stable, in the absence of therapy there is a gradual decline in the cellular immune response in the majority of patients, so that they downgrade to a more lepromatous form of disease. Against this background, spontaneous fluctuations in the immune response are responsible for leprosy reactions. Type 1 reactions are usually 'upgrading' or reversal reactions (RR) and caused by an increase in cell-mediated immunity to the bacilli in dermal macrophages and Schwann cells, leading to inflammation of skin and nerve trunks. They may occur throughout the whole leprosy spectrum, but are most common in borderline patients. Previously, a

downgrading form of type 1 reactions was described in untreated patients, but these are rarely if ever encountered now and will not be discussed further. Type 2 reaction or erythema nodosum leprosum (ENL) is a systematic inflammatory response to the deposition of extravascular immune complexes and occurs in LL and borderline lepromatous (BL) patients. The management of ENL has recently been discussed,<sup>5</sup> and in this review, the management strategies for RRs in the light of the underlying immunopathology will be discussed.

### Prevalence of reversal reactions

Despite the importance of RRs in inducing nerve damage, estimates of their prevalence and incidence are difficult to obtain because of differences in the case definitions used and the patient population surveyed and the retrospective nature of most studies. The epidemiology of RR has been reviewed<sup>6</sup> and, overall, the cumulative prevalence of RR varies from 8 to 33% for all leprosy patients. Common patterns have emerged which are relevant to interpreting the results of RR treatment in different settings.

1. RRs are more frequent in studies conducted in referral centres compared to field trials, reflecting the more advanced disease in referred or self-reporting patients. For example, in centres in Thailand and Nepal 32 and 26.9% of patients developed RR,<sup>7,8</sup> while in a recent field study in Bangladesh, only 8.8% of patients experienced RR.<sup>9</sup>
2. The type of leprosy affects the risk of RR, so that patients with borderline (BB) and BL leprosy have a higher prevalence than borderline tuberculoid (BT) patients. For example, in a field study in Ethiopia, the incidence rate for RR in paucibacillary (PB) leprosy was 13.1/100 patient years at risk (PYAR) compared to 28.4/100 PYAR in multibacillary (MB) patients.<sup>10</sup>
3. Patients with more extensive diseases, as assessed by the number of body areas<sup>7</sup> or pre-existing nerve function impairment (NFI),<sup>10</sup> are more likely to develop RRs leading to further tissue damage, and these patients have a less satisfactory response to therapy.
4. RR can occur at any time during treatment, being present at diagnosis in 2–5% of patients in Africa<sup>6</sup> and 30–47% in Nepal and India.<sup>7,11</sup> The highest risk is during the first year of treatment, with up to 80% occurring in the first 6 months of MDT,<sup>12</sup> but RR can occur later, particularly in BL patients.<sup>13</sup> Importantly, RR may emerge after the completion of MDT. In Ethiopia,<sup>14</sup> 10.2 and 7.8% of BT patients experienced RR before and after 6 months of MDT, respectively, compared to 22.8 and 10.7% of multibacillary (MB) patients before and after 2 years of MDT in India.<sup>15</sup> Both these studies have confirmed the clinical impression that relapses of RR is common, with up to a third of RR patients having recurrences.

### Immunopathology of reversal reactions

Understanding the immunopathology of RR allows the rational use of appropriate therapy. Analysis of the skin lesions from patients in reaction have defined the cellular inflammatory processes,<sup>16</sup> although the factors triggering the characteristic increase in cellular reactivity to *M. leprae*<sup>17</sup> are still poorly understood. The tissues are infiltrated predominantly with CD4<sup>+</sup>αβ T cells, although γδ T cells are also present,<sup>18</sup> with accompanying oedema and

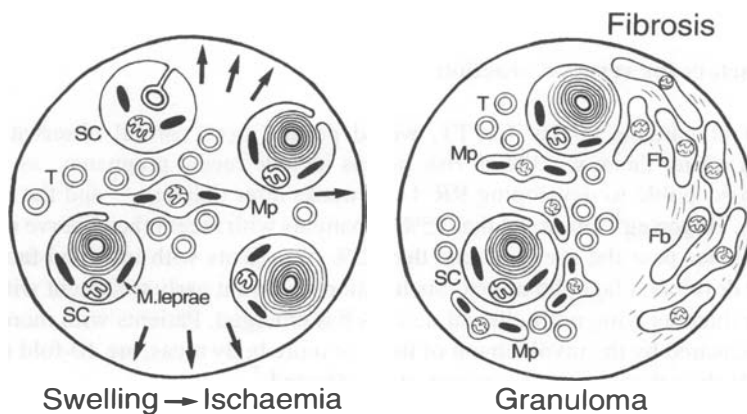


increased expression of adhesion molecules on endothelium and overlying keratinocytes.<sup>19</sup> This influx of lymphocytes and monocytes produces localized delayed type hypersensitivity (DTH) reactions in the skin and nerves. The presence of activated T cells in reactional lesions results in elevated IL-2 receptor levels in blister fluid from lesions.<sup>20</sup> There is a dramatic increase in the number of interferon- $\gamma$  (IFN- $\gamma$ ) expressing cells and levels of IFN- $\gamma$  mRNA in reactional lesions.<sup>21</sup> Analysis of T cell clones derived from skin biopsies before and after the development of RR confirmed the shift to a polarized pattern of cytokine release dominated by IFN- $\gamma$  during RR.<sup>22</sup> Intriguingly, higher levels of mRNA and protein for tumour necrosis factor (TNF) are present in reactional nerves than skin lesions.<sup>23</sup> Nerve function is impaired by the local ischaemia secondary to the compression of perineural blood vessels by the inflammatory oedema and by direct destruction of Schwann cells and axons by the CD4<sup>+</sup> T cell-mediated granulomatous process (Figure 1).<sup>24</sup> With time, post-inflammatory fibrosis leads to irreversible nerve damage. The intensity of the localized inflammation is reflected by raised serum levels for TNF,<sup>25</sup> soluble IL-2 receptors<sup>26</sup> and adhesion molecules.<sup>27</sup> The institution of effective anti-inflammatory therapy rapidly reverses these abnormalities.

### Clinical issues in management

RR may take the form of one or more of the following:

1. Increased inflammation with swelling and erythema in established skin lesions, or in BL and subpolar LL patients, in newly appearing lesions.
2. Acute inflammation in affected nerve trunks with tenderness and pain over the nerve and loss of motor and/or sensory function in its distribution.
3. Recent (<6 months) and/or progressive loss of motor or sensory function in the absence of painful neuritis.



**Figure 1.** Mechanisms of nerve damage in reversal reactions. The influx of T lymphocytes (T) and macrophages (Mp) with resultant oedema causes swelling and secondary ischaemia to the nerve. *M. leprae*-infected Schwann cells (SC) and axons were also destroyed directly by the T lymphocyte-driven granulomatous response to chronic antigenic stimulation. Fibroblasts (Fb) are responsible for post-inflammatory fibrosis which can cause continuing nerve damage after the features of inflammation have subsided.

Education of patients and staff is vital for the early recognition of RR. All newly diagnosed BT, BB and BL patients should be warned of the possibility of RR during treatment and the importance of immediate presentation to enable prompt therapy. Staff should be trained to examine for the features of NFI at each visit, using simple screening procedures for voluntary motor testing (VMT) and sensation. There is considerable variation in the methods used for detecting NFI, and the frequency and care in testing will influence the number of patients detected with NFI. Recent comparisons of different methods for detecting sensory impairment demonstrated that testing with multiple nylon monofilaments or for two-point discrimination were more sensitive than simple pin-prick sensation.<sup>28</sup> The major source of variability between testers was testing skill and experience, emphasizing the importance of staff training. The challenge is to standardize simple and reproducible tests for implementation in field programs so that the majority of leprosy patients will benefit. This is particularly relevant to the management of leprosy patients in general health services where medical staff are less familiar with leprosy complications.

At initial assessment or on serial testing, some leprosy patients will be detected with NFI and no other features of typical RR. There has been confusion as to whether these patients have RR and warrant treatment and the phenomenon has been referred to as 'quiet nerve paralysis' or 'silent neuritis'. In a retrospective analysis of 536 patients in a referral centre, 7% of new patients had silent neuropathy and its incidence rate was 4.1 per 100 PYAR with 75% of episodes occurring in the first year.<sup>29</sup> Although cell-mediated inflammation is probably the major aetiological factor, Schwann cell dysfunction and post-inflammatory fibrosis may also contribute to this silent neuropathy and some studies now identify patients with NFI and RR separately. In a field study in Bangladesh,<sup>9</sup> the incidence rate of NFI was 3.5/100 and 7.5/100 PYAR in PB and MB patients, respectively, compared to rates for 'clinical' RR of 1/100 and 6/100 PYAR, respectively. Further studies are required to define the epidemiological significance and natural history of this group. However, as the motor and sensory function in the affected nerves improves significantly with corticosteroid therapy, inflammatory damage must be a significant factor and these patients can be operationally considered with RR patients for diagnosis and therapy.

### Predictive factors for reversal reaction

Identification of patients at risk for TT, would permit more careful observation of this subgroup and earlier therapy. Clinical risk factors include recent pregnancy, as women are particularly susceptible to developing RR 4–12 weeks after delivery,<sup>13</sup> and the presence of facial plaques. Hogeweg<sup>30</sup> observed that 85% of patients with recent facial nerve damage had significant patches near the eye, and also that 45% of patients with inflamed facial patches subsequently developed lagophthalmos. Such patients warrant early treatment with corticosteroids rather than delaying until clinical neuritis has emerged. Patients with more extensive disease, as measured by the involvement of three or more body areas, are 10-fold more likely to develop RR than those with one or two areas affected.<sup>7</sup>

RRs are a manifestation of increased cell-mediated immunity to *M. leprae*, and although increased serum levels of some cytokines and soluble receptors occur during RR, these cannot be used to predict the onset of RR. The levels of *M. leprae*-specific antibodies correlate with the bacillary load and clinical extent of disease.<sup>31</sup> When borderline leprosy patients had both anti-*M. leprae* phenolic glycolipid IgM antibodies and a positive lepromin

test, as evidence of T cell responsiveness to *M. leprae*, there was a significant increase in the cumulative prevalence of RR to 78% compared to 31% in all borderline patients.<sup>12</sup>

Roche<sup>32</sup> has analysed a cohort of 534 Nepali borderline leprosy patients for risk factors, present at the start of treatment, which predict the development of RR at any stage during therapy. Of the 12 parameters analysed by logistic regression, three were independently associated with a significant risk of subsequent RR. These were the presence of facial lesions [adjusted odds ratio for RR (AOR) = 4.6], involvement of three or more body areas (AOR = 2.2) and positive IgM anti-PGL antibodies (AOR = 1.72).<sup>32</sup>

A final clinical issue is distinguishing between late RR and relapse in patients who have completed MDT, particularly in skin smear negative PB patients. Clinical criteria for differentiating the two have been proposed<sup>33</sup> and skin biopsy where available may assist. However, it may not be possible to differentiate between the two conditions in all patients. Operationally, patients with increased NFI should be treated with a full course of corticosteroids and either MDT, if there is any possibility of relapse in smear positive patients, or a single drug, preferably clofazimine 50 mg daily, in smear negative patients.

### Current management of reversal reaction

The principles of management for RR are maintenance of anti-mycobacterial drugs, effective and prolonged anti-inflammatory therapy, and adequate analgesia and physical support during the phase of active neuritis. It is imperative that MDT is maintained after the onset of RR, as reduction in the antigenic load in skin and nerves removes the target for the T cell-driven inflammation and lessens the propensity to recurrence.

### Corticosteroids

Although there is a paucity of controlled trials assessing therapy for RR, there is a consensus that corticosteroids form the cornerstone of drug therapy. Some authors recommend a staged approach to therapy with a trial of aspirin and/or hydroxychloroquine for 'mild reactions' involving the skin alone without nerve impairment.<sup>13,34</sup> This approach may be counter-productive, as the definition of this subgroup is arbitrary and there are no studies in RR showing that this conservative therapy is effective or prevents progression to nerve damage. It may have arisen from a reluctance to use corticosteroids when this required hospital admission and from an under-estimation of accompanying nerve damage detected by routine clinical examination. For example, 19% of patients with neuritis in one nerve have significant slowing of motor conduction in other clinically unaffected nerves.<sup>35</sup> Moreover, full recovery of nerve function does not occur in a significant proportion of RR patients despite corticosteroid therapy. Therefore the early use of corticosteroids is recommended once there is unequivocal evidence of increased inflammation in established or new skin lesions, NFI of <6 months duration or tender nerves. The earlier effective therapy is instigated, the more likely permanent nerve damage will be prevented.

Corticosteroids modify the course of RRs in a number of ways. They reduce cutaneous and intraneural oedema, leading to a rapid improvement within days in some patients and also reduce post-inflammatory scar formation during the prolonged healing phase.<sup>36</sup> Their chief effect, however, is to suppress the T cell-driven inflammatory response to *M. leprae* antigens

within the skin and nerves. Therefore, immunosuppressive doses of corticosteroids are required for prolonged periods as the reaction will persist whilst the bacillary load gradually falls. Although the optimal doses and duration of therapy have not been established by clinical trials, the recommended regimens reflect these principles. Rose and Waters<sup>13</sup> and Naafs<sup>36</sup> recommend an initial dose of 40 mg of prednisolone (or prednisone). This is sufficient to control most RRs; however, patients with severe NFI and no response after a week may require a higher dose of 60 mg (or 1 mg/kg) and occasionally further increases up to 120 mg to control nerve symptoms. Although some authorities recommend a starting dose of 60 mg prednisolone,<sup>37</sup> field studies with fixed regimens indicate that a dose of 40 mg prednisolone is sufficient to control 85% of RRs.<sup>14</sup> A retrospective comparison of four corticosteroid regimens used in Nepal demonstrated that there were no differences in outcome for regimens starting with dexamethasone 6 mg, prednisolone 30 mg twice daily or prednisolone 60 mg once daily, both reducing over 6 months, or prednisolone 40 mg daily reducing over 4 months.<sup>38</sup> Once there is evidence of improvement, the dose of prednisolone can be reduced at about 5 mg every 1–2 weeks until 20 mg is reached. This is usually continued for some months while more gradual improvement occurs<sup>39</sup> until optimal nerve function has been obtained, and then the drug withdrawn at 5 mg per fortnight. The maximum improvement occurs in the first 3 months, but may continue for up to 6 months.

In ideal circumstances, the length and dose of therapy is individualized based on careful assessment of motor function by VMT and sensitive tests of sensory function, such as the nylon monofilament test.<sup>28</sup> Generally, most BT patients require prednisolone for 4–9 months, BB patients for 6–9 months and BL patients for 6–18 months and even 24 months.<sup>13</sup> One comparative trial demonstrated that prednisolone therapy of longer duration (3–18 months) was superior to short courses of therapy (2 months) for recovery from motor impairment.<sup>40</sup> More recently, 12 weeks of prednisolone therapy for RRs in BB/BL patients was found to be inadequate, with one-third of patients relapsing; however, extension of therapy to 20 weeks resulted in a low recurrence rate.<sup>14</sup>

The recognition that ambulatory therapy with corticosteroids is both effective and safe has been a major advance in the management of RR. Ideally, RR patients should be admitted to hospital, but adherence to such a policy may limit the use of corticosteroids to only a minority of those who require RR treatment. This approach was first demonstrated by Kiran,<sup>41</sup> who observed improvement of 66% of affected nerves in 33 patients treated with prednisolone 25 mg as the initial dose for an average of 5 months. Subsequently, in a larger field study in Ethiopia, standardized courses of 12 weeks and 20 weeks (both starting at 40 mg prednisolone) were used for both RR and ENL reactions in BT and BB/BL patients.<sup>4</sup> Patients with severe ENL, recurrent reactions, deteriorating nerve function despite corticosteroids or associated medical problems were referred for hospital therapy, along with patients in whom late RR could not be distinguished from relapse after completing MDT. Nevertheless, 85% of all RR patients selected by these criteria could be treated at home. Of these 161 patients, 88% showed improvement in nerve function. Even allowing for the exclusion of patients with severe or recurrent reactions, this compares favourably with recovery rates for RR following hospital treatment. These range from 63% for improvement in affected nerves in BT and BL patients receiving a fixed regimen of 6 months prednisolone starting at 40 mg daily<sup>42</sup> to 50% for improvement in neuritic episodes when lower doses of prednisolone were used for shorter periods.<sup>11</sup> Following four regimens of high dose corticosteroids for 4–6 months, the recovery rates varied from 30 to 84%, depending on the nerve involved.<sup>38</sup>

A variety of factors influence the response to corticosteroids. The level of nerve

impairment at the initiation of therapy has a major bearing on outcome. Only 35% of patients with complete anaesthesia and 11% with motor paralysis improved to good function at 3 months, compared to 67% and 55% for patients with moderate impairment.<sup>38</sup> Remarkably similar outcomes were obtained in severe or moderately affected patients after corticosteroids alone or combined medical and surgical treatment.<sup>43</sup> Patients with recent NFI, of less than 6 months duration, demonstrate greater improvement in nerve function than those with old impairment, and this is the usual time limit set for the instigation of reaction therapy in patients with NFI alone.<sup>14</sup> Whilst also observing maximum recovery in those with recent NFI, van Brakel and Khawas<sup>38</sup> did note significant improvement in sensory function after 3 months prednisolone therapy in some patients with NFI of over 6 months duration. Studies are required to determine if this group of responsive patients can be defined more accurately. The leprosy type also affects response and two studies have reported significantly better recovery in BL than in BT patients.<sup>38,42</sup>

There are also differences in the rate of recovery for different nerves. In five separate studies a higher proportion of patients with median nerve damage recovered motor and sensory function than those with ulnar nerve damage.<sup>14,38,41,42,44</sup> Improvement in recent facial nerve paralysis was observed in 70–75% of patients in two studies using standardized corticosteroid regimens;<sup>14,15</sup> however, in another study therapy, this resulted in recovery in only 30% of these,<sup>38</sup> emphasizing the importance of early treatment.

Patients with 'silent neuritis' also respond to corticosteroids. Srinivasan<sup>44</sup> initially observed that in a small group of 25 patients with 'silent neuritis' 75% recovered motor function, 83% in those with paralysis of <13 weeks and 53% when the paralysis was of longer duration. In Nepal, motor and sensory function in nerves affected by silent neuropathy also improved significantly after 3 months of prednisolone therapy.<sup>29</sup> In a recent field study in Bangladesh, 100 patients with recent NFI (<6 months duration) were treated with fixed duration prednisolone (starting dose 40 mg, for 16 weeks) regardless of whether other features of RR were present. Using conservative measures of change (at least 2 points in motor/sensory scores) 65 and 48% of patients showed improvement in sensory or motor function, respectively, and this improvement persisted for >6–8 months after therapy.<sup>46</sup> Therefore patients with NFI alone can be successfully and safely treated with fixed duration therapy in the community, but a significant proportion of patients still fail to respond. The broader application of corticosteroids in ambulatory settings has not been associated with an increase in complications of treatment, but careful pre-assessment for other diseases is important.<sup>47</sup> In particular, infections such as tuberculosis, amoebiasis and strongyloides may be exacerbated by corticosteroid therapy and these should be excluded or treated as necessary.

### Other therapies for reversal reaction

What other therapeutic options are available for RR? Patients with severe neuritis and pain may require hospitalization for parenteral narcotics and splinting of the affected limbs.<sup>34</sup> Clofazimine is an important component of MDT for MB leprosy and may contribute some anti-inflammatory effect.<sup>37</sup> This value is less marked in RR than ENL, and in particular, clofazimine is not effective in the acute phase of RR. Therefore, the dose of clofazimine is usually unchanged during therapy for RR. Clofazimine may have a prophylactic role against RR. In a recent study, borderline leprosy patients considered to be at a high risk for RR, who

received a modified MDT regimen with initial higher doses of clofazimine (300 mg/day compared to 50 mg for the first 12 weeks), experienced a significant reduction in RRs over 2 years (5.7%) compared to that observed in a retrospectively chosen control group (26.1%).<sup>48</sup> This finding warrants a properly controlled prospective trial to determine if this regimen should be more widely implemented.

Azathioprine has been employed both as an immunosuppressant and for its steroid-sparing effect. In a small study, a slightly higher proportion (90%) of patients receiving azathioprine (1 mg/kg) and prednisone for 6 months demonstrated greater motor improvement than those receiving prednisone (77%) or azathioprine (79%) alone.<sup>49</sup> As azathioprine acts slowly and has no effect on intraneural oedema, it should only be used as an adjunct after initial treatment with corticosteroids.

Cyclosporin A is a potent immunosuppressant which acts directly on CD4<sup>+</sup> T cells and has proven benefit in controlling transplant rejection and graft versus host disease. There are case reports of its value in controlling severe steroid-unresponsive RR with the response to cyclosporin (7 mg/kg) being evident at 10 days<sup>50,51</sup> and it may be as effective as corticosteroids in patients with untreated RR. However, its expense limits its availability and use in leprosy endemic countries.

Surgery with decompression of the epineurium of swollen nerves has been previously advocated for patients with persistent nerve pain despite corticosteroid therapy. Although this may improve nerve conduction and function in individual cases, enhanced benefit has not been confirmed in clinical trials. Recent analysis of a randomized comparative trial of corticosteroids and surgical intervention in 39 patients with early neuritis showed that decompression of the ulnar nerve and medial epicondylectomy with medical therapy had no additional benefit over corticosteroids alone at follow-up after 1 or 2 years.<sup>43</sup> Therefore surgery should be reserved for the rare situations of nerve abscess in BT and TT patients or intractable pain despite vigorous immunosuppressive therapy.

### Future approaches to management

Improvement in outcome for nerve function in RR in the immediate future will probably derive from the optimal use of current drugs, particularly corticosteroids, rather than new treatment modalities. The first and most important factor is the early diagnosis of RR or NFI. All descriptive studies confirm long-term nerve function is determined by the duration and level of impairment at commencement of therapy for RR. The development of reproducible tests applicable in the field would aid the early recognition of NFI as would the definition of high risk groups with clinical parameters or simple immunological assays. Nevertheless, the education of both staff and patients to recognise the earliest signs of RR will remain the chief operational factor in the diagnosis of RR.

Second, there is still the need to determine the optimal dose and length of therapy with corticosteroids in RR. Current outcomes are not satisfactory as 20–40% of patients in recent studies still failed to recover lost sensation or muscle power. To this end, a double-blinded placebo-controlled trial is underway in referral centres in India to compare the use of observed regimens of prednisolone starting at 60 mg or 30 mg for 20 or 12 weeks (Waters MFR, personal communication). Any future candidate drugs should then be formally compared with the optimal corticosteroid regimen. In addition, the efficacy of corticosteroids inpatients with NFI of >6 months duration needs to be resolved with prospective studies.

Third, the frequency of RR in some patient populations raises the question as to whether prophylactic anti-inflammatory treatment would reduce the frequency of RR and progressive NFI. A community-based double-blinded trial is planned in Bangladesh to address this question by testing the effect of prednisolone 20 mg daily for 6 months on RR and NFI (W. C. S. Smith, personal communication).

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## Experimental leprosy in rhesus monkeys: transmission, susceptibility, clinical and immunological findings

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**Summary** A total of 46 Rhesus monkeys (RM) was inoculated with *Mycobacterium leprae* (ML) and followed clinically and immunologically for extended periods. Twenty-one (45.7%) of the RM developed leprosy spanning the known leprosy spectrum, with six of 21 (28.6%) having disease in the borderline lepromatous to lepromatous area of the spectrum. RM with paucibacillary forms of leprosy produced predominantly IgG anti-phenolic glycolipid (PGL-I) antibodies and positive lepromin skin test and/or *in vitro* blastogenesis responses; IgM anti-PGL-I predominated in animals with BB-LL leprosy and correlated with negative immune responses to lepromin. IgG anti-PGL-I antibodies persisted in a number of RM for several years without histopathological evidence of leprosy, suggesting possible persisting sub-clinical infection. The data show that RM are a valuable model for the study of leprosy. Eleven of the 46 RM were inoculated with ML from sources infected with simian immunodeficiency virus (SIV), the monkey counterpart to the human immunodeficiency virus (HIV). The possible effect of SIV on the clinical outcome of ML infection could not be determined due to insufficient numbers of animals to yield statistically significant results.

### Introduction

We previously reported that it is possible experimentally to transmit leprosy to rhesus monkeys (RM) (*Macaca mulatta*) and cynomolgus monkeys (CM) (*Macaca fascicularis*).<sup>1–3</sup>

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Prior to those reports, attempts experimentally to transmit leprosy to RM and other macaque species were notably unsuccessful, although many experimental inoculations had been made over approximately a century.<sup>4</sup> In addition to our prior success in experimental transmission of leprosy to a small number of RM and CM, we also observed three feral CM with positive leprosy serology,<sup>3</sup> and more recently, a case of natural leprosy was diagnosed in a wild-caught CM from the Philippines (Canfield *et al.*, personal communication). To our knowledge, this case of natural leprosy and the previous three CMs with positive leprosy serology, which remain under observation, are the only known reported examples of natural leprosy in macaque species. Herein, we report data from experimental inoculations of a total of 46 RM. ML inocula were taken from sooty mangabey monkeys (SMM) with lepromatous (LL) leprosy or from armadillos that had been experimentally inoculated with ML from SMM or human sources. The results extend our earlier observations that RM are susceptible to experimentally induced leprosy, and permit a comparison of the susceptibility of RM to humans and that of other primate species.<sup>1-6</sup>

## Materials and methods

### INOCULATIONS

Lepromata (see Table 1 for sources) were obtained aseptically, minced and homogenized in cold phosphate-buffered saline using a Dounce homogenizer with a 40 MI mortar and teflon pestle (Wheaton Scientific, Millville, NJ, USA), passed through gauze and centrifuged at 200 g for 5 min at 4°C. The acid-fast bacilli (AFB) in the supernatant were counted and morphological indices (MI) determined by the method of Shepard & McCrae.<sup>7</sup> RM were inoculated with ML suspensions by combined intradermal (ID) and intravenous (IV) routes using two ID sites per ear, the tip of the nose, lateral forearms and lateral calves. IV inoculations were made via the saphenous vein.

### CLINICAL OBSERVATIONS

Animals were observed twice daily and examined in detail three or four times per year or more, depending on the status of the animal. Clinical aspects of the disease were recorded at each time of observation. The Ridley-Jopling system was used to classify leprosy histopathologically,<sup>8</sup> with the exception that classification at the paucibacillary end of the spectrum differs slightly in RM from humans. The Ind/TT (indeterminate/tuberculoid) classification has been included to describe a well-defined grossly tuberculoid-appearing

**Table 1.** ML inoculation of RM<sup>a</sup>

No. of RM inoculated	ML origin	ML dose ( $\times 10^{-8}$ )	No. of RM with leprosy
23	SMM/A	1-10	12
11	SMM	1-10	6
12	HU/A	50-1000	3

<sup>a</sup> Abbreviations: ML, *M. leprae*; RM, rhesus monkeys; SMM, sooty mangabey monkeys; Hu, human; and A, armadillo.

lesion with rare AFB, usually in nerves, and minimal to mild perivascular mononuclear inflammatory cell infiltrate. Often the skin lesion is dry, scaly and hypopigmented with alopecia. No histiocytes, epithelioid cells or multinucleated cells are present. TT leprosy by classical histopathological definitions, containing epithelioid and/or multinucleated giant cells together with lymphocytes, is less frequently observed, but does occur in RM. The terms neuritic TT and neuritic Ind/TT are used herein to describe lesions with predominantly neuritic involvement.

#### ELISA

The assays were performed as previously reported.<sup>9-12</sup> Baseline sera were obtained prior to ML inoculations and at intervals after inoculation, and were stored frozen for later ELISA evaluations of ML-specific anti-PGL-I antibody. Natural ML PGL-I was used as antigen (Ag) [specificity for PGL-I was verified using the synthetic glycoconjugate, bovine serum albumin-*O*-(3,6-di-*O*-methyl- $\beta$ -D-glucopyranosyl)-(1-4)-(1-deoxy-2,3-di-*O*-methyl-L-rhamnose) (NDO-BSA), instead of natural PGL-I in all animals; only the natural PGL-I data are reported]. PGL-I and NDO-BSA were provided by Dr Patrick J. Brennan, Colorado State University School of Veterinary Medicine, Fort Collins, CO, USA under NIH contract no. 1-AI-52582.

Briefly, 96-well plates were coated with Ag, washed, blocked with BSA, washed again and reacted with a previously determined optimal dilution of monkey serum (1:150 for IgG and 1:100 for anti-IgM). After incubation and washing, the plates were coated with peroxidase-labelled anti-human IgG or IgM Fc fragment  $\gamma$ - or  $\mu$ -chain-specific Ab diluted according to prior titrations, incubated, washed, reacted with *o*-phenylenediamine + H<sub>2</sub>O<sub>2</sub>, acidified, and optical density (OD) determined at 490 nm on an ELISA reader. Final ODs represent the difference in absorbance between wells containing Ag minus wells lacking Ag but containing all other components. Each reagent in the ELISA was carefully titrated in a checkerboard manner to determine dilutions that would give final OD values between 0.1 and 0.5 OD whenever possible, enabling the utilization of an OD range most sensitive to small changes in OD, so that small changes from sample to sample would have maximal meaning and would accurately reflect longitudinal changes. All sera were assayed together at one time in given experiments to permit accurate relative comparisons. The same batch of peroxidase antibody was used throughout. All experiments were repeated on at least two separate occasions, examining all sera together in each assay. OD values obtained with these precautions were reproducible in a given sample from one assay to another to within  $\pm 0.05$ . Cut-off points for positivity were taken as OD values greater than the mean +2 standard deviations (SD) of the mean of normal RM ( $n = 73$ ). The mean +2 SD cut-off points were 0.018 + 0.114 (0.132) for IgG and 0.017 + 0.094 (0.111) for IgM anti-PGL-I.

Ratios of ELISA values for IgM:IgG were calculated in some instances, as noted in the text, by simply dividing the actual IgG OD value into the IgM OD value, as previously described.<sup>9-12</sup>

#### LEPROMIN SKIN TESTING

RM require 10 times the standard human concentration of lepromin (lepromin A) to induce responses consistently.<sup>6</sup> A concentrated ( $\times 76.9$ ) lepromin A suspension in PBS was prepared by Dr Wayne M. Meyers at the Armed Forces Institute of Pathology for use in these

experiments. The stock was stored frozen and diluted with PBS just prior to use for skin testing. Animals were tested on the shaved lower abdomen by intradermally injecting 0.1 ml of ( $\times 10$ ) lepromin ( $1.6 \times 10^9$  *M. leprae*/ml). The reaction size and characteristics were noted and biopsied on day 28 postinjection (Mitsuda reaction). Animals were skin tested between 1 and 2 years after ML inoculation. Results are according to WHO criteria: -, no reaction; +/- <4 mm; +, 4-6 mm; ++, 7-10 mm and + + +, >10 mm.

#### BLASTOGENESIS

Heparinized blood was used to prepare buffy coats which were centrifuged on Ficoll/Hypaque, washed and suspended in RPMI-1640 + 5% heat-inactivated human AB serum (HuABS) and penicillin/streptomycin. The mononuclear cell (MNC) fraction was used for *in vitro* blastogenesis studies, using an antigen the same lepromin as that used for skin testing. A stock suspension of  $\times 76.9$  lepromin was prepared for blastogenesis by 1:50 dilution into RPMI-1640 + 5% HuABS. A final concentration of 1:20,000 of the 1:50 stock per well reproducibly gave optimal stimulations in preliminary testing and was used throughout this study. U-bottom 96-well microtitre plates were used. Aliquots of  $2 \times 10^5$  MNC per well were incubated at 37°C in 5% CO<sub>2</sub> in triplicate for 5 days with lepromin or media prior to pulsing for 4 h with 1  $\mu$ CI of <sup>3</sup>H-thymidine/well. Thereafter, cells were washed and harvested on a cell harvester and quantified by scintillation counting. Stimulation indices (SI) were determined by subtracting backgrounds from all values and dividing the media control (C) values into the lepromin-stimulated experimental (E) values ( $SI = E/C$ ). Positive SIs were greater than the mean +2 SD of the response of normal RM. *In vitro* lepromin blastogenesis studies were completed immediately prior to lepromin skin testing. The mean +2 SD cut-off value for lepromin blastogenic responses was  $1.66 + 3.12$  (4.78).

## Results

#### CLINICAL

A total of 46 RM were inoculated with ML from a variety of sources over a period of 11 years (Table 1); 21 (45.7%) developed histopathologically documentable leprosy, including nine indeterminate (Ind)/tuberculoid (TT) (Ind/TT), four neuritic TT, two TT, two borderline (BB), one borderline lepromatous (BL) and three lepromatous (LL) forms. Thirty-four of the 46 RM received by combined IV/ID routes a titrated range of  $1 \times 10^8$  to  $1 \times 10^9$  SMM-origin ML [either after subpassage through armadillos (23 RM) or direct from a lepromatous SMM (11 RM)]. An additional 12 RM received titrated amounts ranging from  $5 \times 10^9$  to  $1 \times 10^{11}$  armadillo subpassaged human ML by similar combined IV/ID routes (Table 1). The MIs of the various inocula ranged from 7 to 21%.

These inoculations were performed before it was learned that most captive SMM carry simian immunodeficiency virus (SIV) asymptotically and that SIV is transmissible to other primates experimentally or by contact with SIV-contaminated body fluids such as ML inoculum prepared from the tissues of SMM.<sup>14,15</sup> The ML inoculum was taken from SMM found to be SIV-positive.<sup>15</sup> Although no systematic approach was taken to investigate the role of SIV in leprosy in these studies, observations regarding the SIV status of the ML inocula are summarized for clarification (Table 2). SIV does not cause demonstrable AIDS-like disease in armadillos; virus-culture and PCR studies show that tissues from armadillos inoculated

**Table 2.** Clinical results of RM inoculated with SIV+ or SIV- ML<sup>a</sup>

	RM inoculated	Leprosy +		BB-LL leprosy	
	no.	no.	(%)	no.	(%) <sup>b</sup>
SIV+	11	6	(54.5)	3	(50)
SIV-	35	15	(42.8)	3	(20)
Totals	46	21	(45.7)	6	(28.6)

<sup>a</sup> ML contaminated with SIV (SIV+) or uncontaminated (SIV-).<sup>b</sup> Percentage of leprosy cases.

with SIV + ML are negative for SIV and SIV-derived RNA (Murphey-Corb. M. and Gormus, B. J., unpublished observations).

#### IMMUNOLOGICAL CORRELATIONS

As we have previously documented in other primate species, the presence of IgM anti-PGL-I serum antibody correlated longitudinally with susceptibility to LL forms of leprosy (no. 1, F185; no. 2, A125; no. 3, F179 and no. 7, F187, Table 3) with only rare exceptions (no. 3, B465, Table 3). RM F179 (no. 3) initially developed Ind/TT-type leprosy and, at that time, had only IgG anti-PGL-I antibody in significant quantities (IgM:IgG ELISA ratio <1), but later the disease evolved into LL-type leprosy along with an evolution towards predominantly IgM anti-PGL-I (IgM:IgG ELISA ratio >1 (Table 3). This pattern of a high IgM:IgG anti-PGL-I serum antibody ratio in multibacillary leprosy-susceptible animals was previously observed in SMMs<sup>9,10,12</sup> chimpanzees<sup>16</sup> and RM A125.<sup>6</sup> RM F187 (no. 7, Table 3) initially developed LL leprosy with predominantly IgM anti-PGL-I serum antibody (IgM:IgG ratio >1), but eventually upgraded to Ind/TT leprosy involving predominantly dermal nerves (neuritic leprosy). The evolution of disease in F187 from LL to Ind/TT leprosy was paralleled by a longitudinal evolution in the ELISA-determined anti-PGL-I antibody isotype from predominantly IgM to IgG (Table 3). Many ML-inoculated RM failed to develop clinical symptoms of leprosy in the presence of significant IgG and absence of IgM anti-PGL-I antibody (nos 15–25, Table 3). The persistence of viable ML in the absence of clinical signs of infection in many of these RM was suggested by longitudinally stable high levels of anti-PGL-I IgG antibody, as illustrated in Figure 1a–c by RM's D553, D739 and F183 (nos 21–23, Tables 1 & 3). These longitudinal IgG anti-PGL-I profiles are very similar to RM with paucibacillary leprosy. Of the 38 animals studied immunologically, persisting IgG anti-PGL-I antibody levels were observed in the absence of IgM anti-PGL-I antibody in 13 of the 24 RMs lacking clinical signs of leprosy (Table 3).

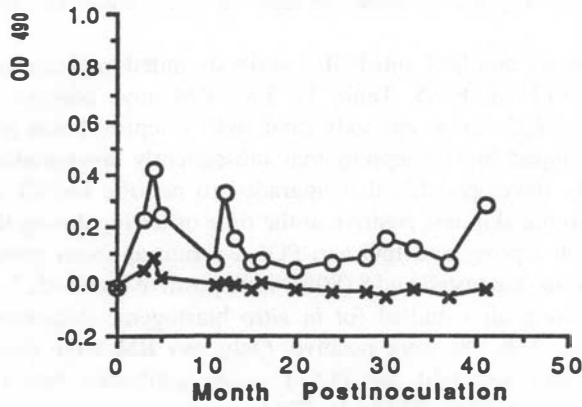
Of 24 RM that failed to develop leprosy, 19 were examined both for anti-PGL-I antibody and for lepromin skin test responses (Table 3). Eleven (nos 26, 28–33, 35 & 36–38) of the 19 (57.9%) failed to produce greater than cut-off levels of either IgM or IgG serum antibody to PGL-I (Table 3). Of these 11 antibody-negative, leprosy-negative RM, eight were lepromin skin tested and six of the eight (75%) were skin test positive (the remaining two were +/-). Seven of the eight antibody-negative, leprosy-negative, skin test-positive RM were also observed for *in vitro* blastogenic responsiveness to lepromin and only one (14.3%) (F719, no. 36, Table 3) was positive. Among the 13 leprosy-negative IgG anti-PGL-I positive group,

**Table 3.** Relationship in RM between clinical leprosy and anti-PGL-1 antibody, lepromin skin test results and *in vitro* blastogenic responses to lepromin

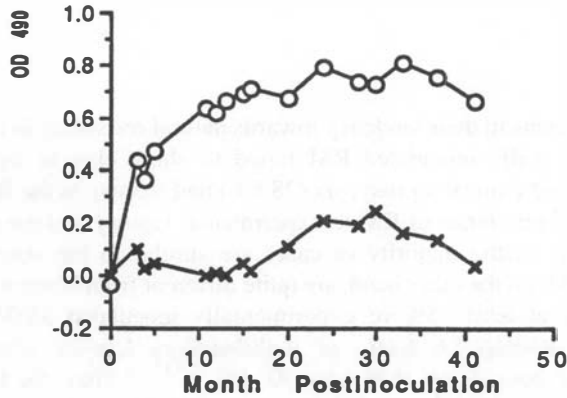
No.	Animal number	Anti-PGL-1 Ab Isotype		Clinical status	Lepromin reactivity		SIV status
		IgM	IgG		ST <sup>a</sup>	Blastogen <sup>b</sup>	
1	F185	+	+	LL <sup>c</sup>	—	—	—
2	A125	+	—	LL	—	ND <sup>d</sup>	—
3	F179	+	+	Ind/TT, LL <sup>g</sup>	+ <sup>e</sup>	—	—
4	A491	—	+	BB	+++	ND	+
5	E423	—	+	BB	++	+	+
6	8664	—	+	BL	—	ND	+
7	F187	+	+	LL, Ind/TT, neuritic <sup>h</sup>	+	—	—
8	B539	—	+	TT, neuritic	ND	ND	—
9	B465	+	+	Ind/TT	+	+	—
10	A749	—	+	Ind/TT	+	—	—
11	F383	—	+	Ind/TT	+	—	—
12	C406	—	+	Ind/TT	—	—	+
13	B988	—	—	Ind/TT	—	ND	+
14	F714	—	—	Ind/TT	+++	—	—
15	B630	—	+	NVL <sup>f</sup>	+	++	—
16	B685	—	+	NVL	+++	++	—
17	B614	—	+	NVL	+++	+	—
18	B960	—	+	NVL	+++	+++	—
19	B748	—	+	NVL	+	ND	+
20	B845	—	+	NVL	+	ND	+
21	D553	—	+	NVL	+++	—	—
22	D739	—	+	NVL	+++	—	+
23	F183	—	+	NVL	ND	ND	—
24	D449	—	+	NVL	+	—	+
25	E118	—	+	NVL	+	—	+
26	B245	—	—	NVL	ND	ND	—
27	B347	—	—	NVL	ND	ND	—
28	A766	—	—	NVL	ND	ND	—
29	B784	—	—	NVL	ND	ND	—
30	B849	—	—	NVL	+	—	—
31	D436	—	—	NVL	+	ND	—
32	D505	—	—	NVL	+/-	—	+
33	F704	—	—	NVL	+	+/-	—
34	F915	—	—	NVL	+++	—	—
35	F596	—	—	NVL	+/-	—	—
36	F719	—	—	NVL	+++	+	—
37	F688	—	—	NVL	+	—	—
38	F762	—	—	NVL	+	—	—

<sup>a</sup> ST, skin test (results according to WHO criteria).<sup>b</sup> Blastogen, blastogenesis SI.<sup>c</sup> LL, lepromatous leprosy; BB, borderline leprosy; Ind/TT, indeterminate/tuberculoid leprosy and TT, tuberculoid leprosy.<sup>d</sup> ND, not determined.<sup>e</sup> + + +, +, +/-; Lepromin skin test results 27 months after the first inoculation (+ + +); 3 months after a second inoculation (+) and 29 months after the second (SIV contaminated) inoculation (+/-).<sup>f</sup> NVL, no visible leprosy.<sup>g</sup> The initial disease in F179 was in the Ind/TT area of the spectrum (skin test and blastogenesis determinations were done during this period), but by approximately 8 years PI the disease evolved to the LL type.<sup>h</sup> The disease in F187 was initially LL (skin testing and blastogenesis were done at this time) but evolved to Ind/TT type involving dermal nerves.

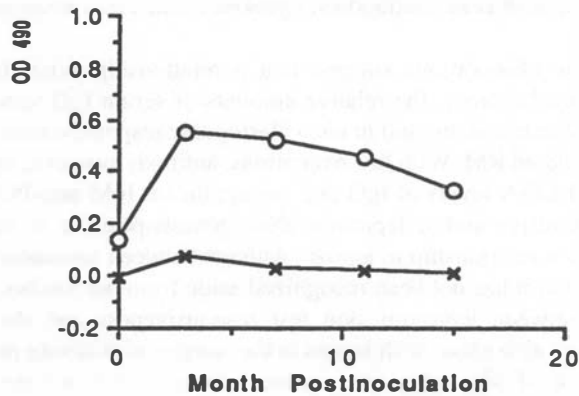
## a. D739



## b. D553



## c. F183



**Figure 1.** ELISA-determined anti-PGL-I IgM (x) and IgG (o) serum antibody profiles preinoculation and longitudinally after *M. leprae* inoculation in RM D739 (a), D553 (b) and F183 (c).

11 were lepromin-skin tested and 100% were positive. Of these 11 RM, nine were also studied for *in vitro* blastogenic responses to lepromin and four (44%) responded positively (Table 3).

RM with LL leprosy and IgM anti-PGL-I antibody failed to give positive lepromin skin tests (nos 1 & 2, A125 & F185, Table 3). Two RM gave positive lepromin skin test responses at the time IgG serum antibody (and Ind/TT leprosy) was present: no. 3, F179 which initially developed Ind/TT leprosy that subsequently downgraded to LL and no. 7, F187 which initially developed LL that upgraded to neuritic Ind/TT (Table 3). Both of these RM were lepromin skin test positive at the time of testing during their Ind/TT disease period. Ten RM with leprosy and IgG anti-PGL-I serum antibody (nos 3–7, 9–12 & 34, Table 3) were lepromin skin tested and 8 (80%) were positive (nos 3–5, 7, 9–11 & 34). Seven of these eight RM were also studied for *in vitro* blastogenic responsiveness to lepromin and two (28.6%, nos 3 & 38) were positive. Only two RM were found to have clinical leprosy with both IgG and IgM anti-PLG-I serum antibodies below the cut-off point (no. 13, B988 and no. 14, F714, Table 3). F714 was lepromin skin test-positive and B988 was lepromin-negative.

## Discussion

RM are similar to humans in their tendency towards natural resistance to leprosy in that 25 of 46 (54.3%) experimentally inoculated RM failed to show clinical signs of leprosy and of the 21 that developed clinical leprosy, six (28.6%) had leprosy in the BB-LL region of the spectrum. The natural resistance of RM to experimental leprosy and the propensity towards paucibacillary leprosy in the majority of cases are similar to the observations in human leprosy. Captive SMM, on the other hand, are quite different from humans and RM in leprosy susceptibility in that at least 75% of experimentally inoculated SMM (and two known natural cases<sup>3,13,17</sup>) develop LL forms of multibacillary leprosy when inoculated with the minimal infective dose of less than  $4.8 \times 10^7$  ML.<sup>3,9,11,12</sup> Thus, the RM appears to be a more appropriate non-human primate model than the SMM for the study of leprosy. Both SMM and RM are similar to humans phylogenetically and human immunological reagents cross-react with lymphoid cells, antibodies, cytokines and other immune elements in both species.<sup>3,6,9–13,18–23</sup>

The immunologic observations suggest that a relationship exists between resistance/susceptibility to clinical leprosy, the relative amounts of serum IgG versus IgM anti-PGL-I antibody, lepromin skin test status and *in vitro* blastogenic responsiveness to lepromin among experimentally inoculated RM. With few exceptions, antibody-positive, leprosy-resistant RM which had positive ELISA levels of IgG and no significant IgM anti-PGL-I antibody, were lepromin skin test-positive and/or lepromin blastogenesis-positive *in vitro*. It is generally accepted that there is a relationship in leprosy patients between resistance and lepromin skin test responsiveness, but it has not been recognized aside from our studies, to our knowledge, that a correlation between lepromin skin test responsiveness and the humoral immune response compartment also exists with regard to the isotype of antibody produced in response to the PGL-I antigen of ML. This relationship between CMI and the humoral immune compartment may be direct or coincidental, but appears likely to depend at least partially upon common or overlapping lymphokine pathways between the humoral and the CMI compartment. Thus, successful CMI in the RM, as manifested by skin test responsiveness



and/or blastogenesis against ML antigens, correlates with the production of IgG but not IgM isotype serum antibody against the PGL-I ML cell wall antigen.

It is noteworthy that we observed that positive skin test responses do not always directly correlate with positive *in vitro* blastogenic responses to ML antigens, so that lack of an effective clinical response could be due to a failure of effector cells to migrate into the local dermal sites of ML growth or to a failure of effector cells to respond appropriately to ML antigens or both.

These and our previous observations in RM, SMM and chimpanzees suggest that CMI and anti-PGL-I IgG/IgM serum antibody production may be under similar or overlapping feedback controls, with IgG isotype being associated with increased and IgM isotype being associated with decreased anti-ML CMI.<sup>6,9,10,12,16</sup> We do not imply that the antibody necessarily plays a direct role in the CMI responses or even in the elimination of the pathogen. We only note that an association between the anti-PGL-I antibody compartment and the anti-ML CMI compartment exists. In fact, 58% of the leprosy-negative RM (11 of 19 studied) failed to produce significant levels of either IgG or IgM anti-PGL-I antibody responses; 67% of these antibody-negative, leprosy-negative RM that were also lepromin skin tested were lepromin-positive. Thus, it is possible to mount an effective CMI response to ML antigens capable of eliminating the pathogen in the absence of a significant detectable antibody response. It would be difficult or impossible to document such a relationship in humans, however, because of the long unrecognized incubation period and the fact that most of the changes noted in the immune status of non-human primate models take place early after infection and evolve to a less dynamic stage by the time clinical disease is diagnosed.

We demonstrated that some RM maintain an elevated or fluctuating level of IgG anti-PGL-I antibody that can persist for years in the absence of visible clinical disease. These RM are believed to harbour viable 'persister' ML. Thus, it would appear that the persistence of anti-PGL-I antibody is a good indicator of the persistence of viable ML in the host; so long as that antibody is of the IgG isotype, little or no clinical progress of the disease appears likely. Such animals usually give a strong positive lepromin skin test and/or *in vitro* blastogenic responses.

Finally, the main strength of the RM model may be in the study of the immunological changes in conjunction with clinical implications in the understanding of the pathogenesis of leprosy early postinfection, a time period which is nearly impossible to study in humans. We do not know the significance of the association between the serum anti-PGL-I responses and the relative degree of susceptibility to the spectral forms of leprosy. It appears important to study this phenomenon in greater detail, however, since it may lead to the further definition of the role of lymphokines and other cytokines in overlapping immune pathways. Such information could lead to the discovery of as yet unrecognized and undefined areas of potential immunoprophylactic and/or immunotherapeutic intervention in leprosy and other mycobacterial diseases.

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## ***In vitro* studies on extracellular matrix production by *M. leprae* infected murine neurofibroblasts**

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**Summary** Fibroblasts and a host of macrophage secretory products have been implicated in a number of diseases where excess extracellular matrix (ECM) deposition is the main pathological feature. Fibrosis characterized by excessive deposition of collagen also contributes to the irreversible nerve damage observed in leprosy. Since *M. leprae* are seen within neurofibroblasts (Nf) in the advanced stages of the disease and macrophages form a common infiltrating cellular constituent of leprosy nerves at all stages, secretion of ECM proteins by Nf was studied, *in vitro* following infection with *M. leprae* and in the presence of macrophage secretory products. These studies were compared in cells derived from two strains of mice, Swiss White (SW) and C57BL/6, as they differ in their response to *M. leprae* infection and parallel those observed in lepromatous and tuberculoid patients, respectively. On infection with *M. leprae*, Nfs showed a decrease in secretion of collagen type IV in SW and type I in C57BL/6 strain. Macrophages caused a further decrease in the secretion of collagen types affected by *M. leprae* infection *per se*, while the other collagen types, viz. I and III in SW strain and III and IV in C57BL/6 strain, were unaffected. This study indicates that neural collagenization in nerves in advanced leprosy may be of Nf origin. However, unlike other diseases with excess collagen deposition, ECM proteins produced by Nfs in response to nerve damage may not be of prime importance in the progression of leprosy neuropathy and occur as a general response to loss of cellular content in leprosy nerves.

### **Introduction**

Neurofibroblasts (Nfs) form a major portion of the non-neuronal cell population that is present in the peripheral nerve. They are present scattered throughout the epineurium and endoneurium,<sup>1</sup> and cells forming the perineurium have also been shown to be of fibroblast origin.<sup>2</sup> In conjunction with Schwann cells, fibroblasts provide the extracellular matrix (ECM) components of the nerve,<sup>3,4</sup> which consists prominently of collagen and fibronectin.

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Fibroblasts are actively involved in general tissue repair, during which they proliferate and secrete ECM proteins, proteases and protease inhibitors.<sup>5-7</sup> Following nerve injury, the neural fibroblast population increases, and recent work indicates that along with macrophages, they may also be important in the removal and reutilization of myelin debris due to their ability to express apolipoproteins.<sup>8</sup> Their importance in nerve repair is further highlighted by their ability to reinstate the competence of dystrophic mouse-derived Schwann cells to produce and assemble normal basal lamina,<sup>9,10</sup> while ECM proteins produced by them are known to induce neurite outgrowth.<sup>11</sup> Like other tissues, peripheral nerve repair is also aided by macrophages,<sup>12</sup> and a number of macrophage factors have been shown to regulate ECM metabolism in fibroblasts. However, fibroblasts and macrophages have also been implicated in the initiation and progression of a number of diseases where excess ECM deposition is the main pathological feature.<sup>13</sup>

Fibrosis characterized by excessive deposition of collagen, and extensive proliferation of Nfs contributes to the irreversibility of leprous nerve damage.<sup>14</sup> Histopathological observations show evidence of active ECM accumulation in both early and late leprous nerve.<sup>15,16</sup> However, in the early stages, *M. leprae* is seen only within Schwann cells. In addition, in the early stages, fresh collagen pockets are observed around involved fibres<sup>17</sup> and *in vitro* studies demonstrate an increase in collagen production by *M. leprae*-infected Schwann cells.<sup>18</sup> This indicates perturbed Schwann cell ECM metabolism as the contributing factor at this stage of the disease. It is only in the later stages of nerve damage, when a general increase in the interstitial collagen is observed, that *M. leprae* are seen within fibroblasts. This, in addition to the fact that macrophages are the common infiltrating cellular constituent of the leprous nerve, indicates a strong possibility for *M. leprae* and macrophage mediated and regulated accumulation of ECM proteins by Nfs. Therefore ECM protein secretion by Nf cultures was studied following infection with *M. leprae* and in the presence of macrophage secretory products, to determine the contribution of these cells in leprous nerve fibrosis and collagenization. These studies were compared in cells derived from two strains of mice, Swiss White (SW) and C57BL/6, as they differ in their nerve pathology,<sup>19</sup> Schwann cell<sup>18,20,21</sup> and macrophage functions<sup>22</sup> in response to *M. leprae* infection. The macrophage responses to *M. leprae* infection in SW and C57BL/6 mice in particular parallel those observed in macrophages from lepromatous and tuberculoid patients, respectively.<sup>22</sup>

## Materials and methods

### MICE

The mouse strains used in this study were SW and C57BL/6. Nfs were obtained from 2- to 3-day postnatal mice, and 3- to 4-month-old animals were the source for peritoneal macrophages.

### CELL CULTURE PREPARATION

#### *Dissociated Nf cultures*

Cultures were essentially obtained by a modified method of Brockes *et al.*<sup>23</sup> Briefly, sciatic and brachial plexus nerves were collected under aseptic conditions, chopped finely and treated with a 1:1 solution of 0.25% trypsin and 0.05% collagenase (Sigma, USA). They were

dissociated by triturating through a 23G needle and plated on culture dishes in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, USA) supplemented with 10% fetal calf serum (FCS) (Gibco, USA) and antibiotics, penicillin and streptomycin. For determination of proteins secreted into the culture medium, the cells were plated at a density of 300,000 cell/55 mm Petri dish. The cultures were incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> and maintained for 7–9 days. By the end of this period, Nfs in the culture medium grew Schwann cells. As determined by staining with S-100, a Schwann cell marker, the cultures contained 15% of Schwann cells, indicating 85% of cells to be Nfs. The remaining cells were a mixture of Schwann cells and neuroepithelial cells. Seven- to 9-day-old cultures were used for infection with *M. leprae*.

#### *Peritoneal macrophages*

Macrophages were obtained by irrigating the mouse peritoneal cavity with Minimum Essential Medium (MEM). The cells thus obtained were plated on 55 mm Petri dishes ( $5 \times 10^6$  cells/plate) in medium consisting of MEM supplemented with 10% FCS and penicillin. Twenty-four hours later, the cultures were washed free of non-adherent cells and maintained for an additional 5–6 days before infection with *M. leprae* or activation with lipopolysaccharide (LPS).

#### SOURCE OF *M. LEPRAE* AND INFECTION OF CELL CULTURES

*M. leprae* was derived from liver and spleen biopsies obtained from experimentally infected armadillos (supplied by Dr E. Storrs, Florida Institute of Technology). The biopsy was aliquoted into small pieces and stored at –70°C until use. To purify *M. leprae* from biopsy, the tissue was homogenized in DMEM until a smooth homogenous suspension containing no observable tissue pieces was obtained. The suspension was centrifuged at 1000 rpm for 10 min to minimize tissue contamination. The supernatant, containing the *M. leprae*, was further subjected to centrifugation at 4000 rpm. The pellet thus obtained was diluted with DMEM, and *M. leprae* counts of solidly stained bacilli were obtained with Zeil Nelson Carbol Fuchsin (ZNCF) stain.<sup>24</sup> Viability of *M. leprae* in the suspension was determined by the FDA-EB staining method<sup>25</sup> and was generally in the range of 80%. The *M. leprae* suspension was stored at 4°C and used within a week, as the viability of *M. leprae* was well maintained within this time span.

Nf and macrophage cultures were infected for 24 h with  $10^6$  *M. leprae*/ml. As control, Nf cultures fed with heat-killed (autoclaved for 20 min at 120°C at 15 lb pressure) *M. leprae* for 24 h were also included in the study. After 24 h the extracellular bacilli were washed off and the cultures fed with DMEM supplemented with 10% FCS. The bacillary load in Nfs was determined by subjecting cultures on coverslips to ZNCF staining to stain *M. leprae* and 1% methylene blue to stain Nfs. On average, 92% of Nfs were infected after 24 h of infection with *M. leprae* and showed an average bacterial load of 7.5 bacilli/cell.

#### ACTIVATION OF MACROPHAGES WITH LIPOPOLYSACCHARIDE (LPS)

LPS (2 µg/ml) (Sigma, USA) was added to macrophage cultures for 2 h, after which excess LPS was washed off. Cultures were maintained for a further 24 h in MEM supplemented

with 10% FCS and penicillin following which the conditioned medium was collected and used.

#### ADDITION OF MACROPHAGE CONDITIONED MEDIUM TO Nf CULTURES

Conditioned medium from uninfected, 3 day post-infected and LPS activated macrophage cultures was centrifuged at 4000 rpm to remove extracellular bacilli and cellular debris. They were added immediately to Nf cultures with an equal volume of DMEM supplemented with 10% FCS and antibiotics and maintained for 72 h.

#### ESTIMATION OF SECRETORY PROTEINS

Nf cultures in 55 mm petri-dishes were pulsed with  $^{14}\text{C}$ -leucine (5- $\mu\text{Ci}/\text{plate}$ ) (Amersham, Denmark) in 2.5 ml leucine-free DMEM supplemented with 10% FCS and antibiotics.  $^{14}\text{C}$ -Leucine was added to 3 day, post-*M. leprae* infected, heat-killed *M. leprae* fed HKML and corresponding uninfected Nf cultures. Thereafter, spent medium was collected every 72 h and cultures replaced with fresh medium containing  $^{14}\text{C}$ -leucine for a period of 12 days.

Proteins in 2 ml of the collected conditioned medium were precipitated by treating twice with 20% trichloroacetic acid. The protein precipitate was dissolved in 1 N NaOH, added to 10 ml of Bray's fluid and radioactivity determined in a scintillation counter. Part of the culture supernatants was used to determine levels of secreted collagen and fibronectin by ELISA.

#### ESTIMATION OF LEVELS OF COLLAGEN TYPE I, III AND IV

Conditioned medium (50  $\mu\text{l}$ ) from Nf culture was coated at a protein concentration of 200  $\mu\text{g}/\text{ml}$  in 96 well Nunc Maxisorb (Denmark) plates at 4°C for 72 h. Similar quantities of DMEM supplemented with 10% FCS was coated as a negative control. The wells were washed off and non-specific blocking carried out with 0.1% BSA in phosphate buffered saline (PBS) at 37°C for 1 h. Then 50  $\mu\text{l}$  of polyclonal goat antibodies to type I, III, and IV (Sera-lab, code 1310, 1330 and 1340, respectively) collagen was added at a dilution of 1:1000 and the mixture incubated for 3 h at 37°C. After washing off excess antibodies with PBS, 50  $\mu\text{l}$  of horseradish peroxidase (HRP) (Dako, Denmark) labelled anti-goat antibodies was added at a dilution of 1:2000 and incubated for 45 min at 37°C. Following extensive washes with PBS, the plates were incubated with 50  $\mu\text{l}/\text{well}$  of substrate (4 mg of *o*-phenylenediamine with 4  $\mu\text{l}$  of hydrogen peroxide) for 15 min at 37°C and reaction was stopped by adding 50  $\mu\text{l}/\text{well}$  2.5 N  $\text{H}_2\text{SO}_4$ . The optical density (OD) of the coloured product was read at 492 nm in an ELISA reader.

#### ESTIMATION OF FIBRONECTIN

Conditioned medium at a protein concentration of 20  $\mu\text{g}/\text{ml}$  was coated in 96-well Nunc Maxisorb plates at 4°C for 72 h. DMEM supplemented with 10% FCS at a similar protein concentration was coated as a negative control. Preblocking was done with 0.1% BSA for 1 h at 37°C. Monoclonal antibody to fibronectin (Seralab MAS 224) was used at a dilution of 1:2000 in PBS and incubated at room temperature overnight. After washing off excess antibody, HRP labelled anti-mouse antibody was added at a dilution of 1:1000 and incubated

at 37°C for 45 min. Following extensive washes with PBS, the plates were incubated with 50 µl/well of substrate (4 mg of *o*-phenylenediamine with 4 µl of H<sub>2</sub>O<sub>2</sub>) for 15 min at 37°C and reaction was stopped by adding 50 µl/well of 2.5 N H<sub>2</sub>SO<sub>4</sub>. The OD of the coloured product was read at 492 nm in an ELISA reader.

#### STATISTICAL ANALYSIS

Statistical significance was determined by the Student's unpaired *t*-test. Differences between two variables with significance of  $p < 0.05$  were considered significant.

## Results

#### RESPONSE OF Nfs TO *M. LEPRAE* INFECTION

##### *Total protein secretion*

Infection with *M. leprae* or feeding with heat-killed *M. leprae* for 6 days had no effect on secretion of <sup>14</sup>C-leucine labelled proteins by Nfs from both SW and C57B1/6 mice (Table 1). As the age of cultures increased, the amount of radiolabelled proteins secreted by Nfs decreased steadily. However, the trend of protein secretion by infected Nfs versus uninfected Nfs remained unchanged over a infection period of 12 days (Table 1).

##### *Secretion of collagen types I, III, and IV*

Nfs from both strains of mice responded with a decrease in collagen secretion on infection with viable *M. leprae* for 6 days. In the SW strain, the decrease was in collagen type IV, and in the C57B1/6 strain, the decrease was observed in the levels of collagen type I ( $p < 0.05$ ). Secretory levels of the other collagen types were unaffected by infection. Feeding cultures with heat-killed bacilli had no effect on the secretion of any of the collagens studied (Figure 1).

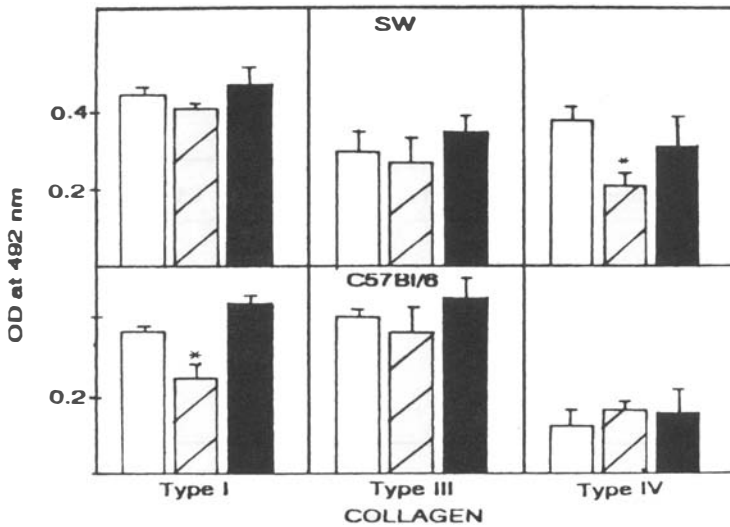
**Table 1.** Secretion of <sup>14</sup>C-leucine labelled proteins by neurofibroblasts. Conditioned medium from uninfected and *M. leprae* infected Nf cultures, pulsed with <sup>14</sup>C-leucine in leucine-free DMEM with 10% FCS, was collected every 72 h and radioactive counts of trichloroacetic acid precipitated proteins were determined. Values given are mean cpm of two independent experiments

Days	SW		C57B1/6	
	Uninfected (cpm)	Infected (cpm)	Uninfected (cpm)	Infected (cpm)
6	97,258	102,091	170,184	169,367
9	2,721	2,204	35,311	39,061
12	982	998	1,880	1,610

#### SECRETION OF FIBRONECTIN

Following infection with *M. leprae* for 6 days, secretion of fibronectin by Nfs was reduced





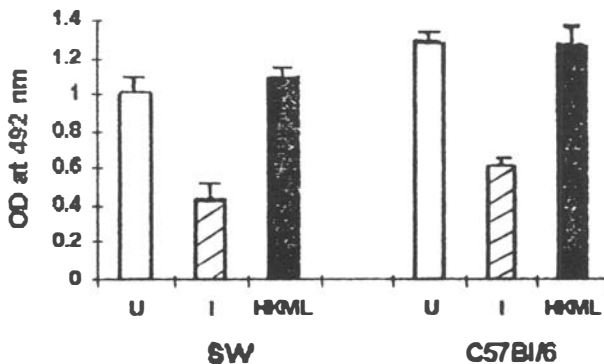
**Figure 1.** Levels of collagen type I, III and IV secreted by uninfected Nf cultures (□) were compared with Nf cultures that were infected with *M. leprae* (▨) or fed with heat-killed (■) *M. leprae* for 6 days in SW and C57Bl/6 strains of mice. Values indicated are mean  $\pm$  SD of three independent experiments performed in triplicates (\* $p < 0.05$  as compared to uninfected Nf cultures).

( $p < 0.01$ ) in both SW and C57Bl/6 mice. Feeding cultures with heat-killed *M. leprae* had no effect on secretion of fibronectin (Figure 2).

#### RESPONSE OF NFs TO MACROPHAGE SECRETORY PRODUCTS

##### Total protein secretion

Nfs from the two strains responded differently to macrophage secretory products. *M. leprae*-infected SW Nfs showed decreased protein secretion on exposure to conditioned medium



**Figure 2.** Levels of fibronectin secreted by uninfected Nf cultures (U) were compared with Nf cultures that were infected with *M. leprae* (I) or fed with heat-killed (HKML) *M. leprae* for 6 days. Values indicated are mean  $\pm$  SD of three independent experiments performed in triplicate.

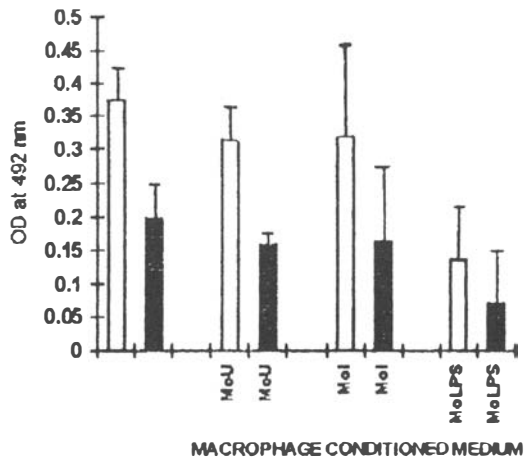
**Table 2.** Secretion of <sup>14</sup>C-leucine labelled proteins by neurofibroblasts in presence of macrophage conditioned medium. Uninfected (U) or viable (I) *M. leprae* infected Nf cultures were exposed for 72 h to conditioned medium (CM) from uninfected (MoU), 3 day post-infected (MoI) or LPS (MoLPS) activated macrophages cultures. Values are mean cpm of two independent experiments

Nf culture + macrophage CM	SW (cpm)	C57Bl/6 (cpm)
U	98,473	159,374
I	97,901	158,290
U + MoU	106,875	119,761
I + MoU	82,115	119,172
U + MoI	102,090	126,371
I + MoI	63,157	127,695
U + MoLPS	75,369	97,255
I + MoLPS	57,394	112,579

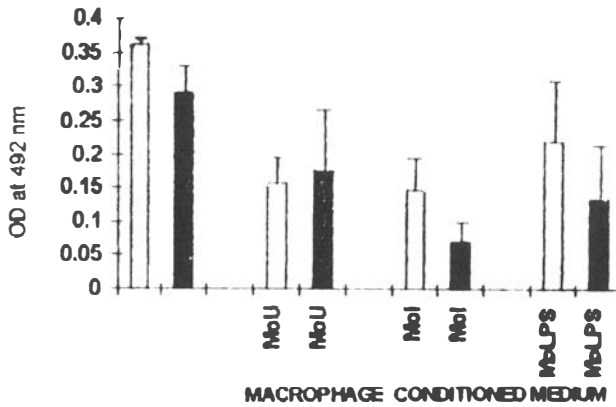
from infected and LPS activated macrophages, while conditioned medium from uninfected macrophages had no effect. In contrast, C57Bl/6 Nfs were refractory to the actions of uninfected, infected and LPS activated macrophage conditioned medium (Table 2).

*Secretion of collagens type I, III and IV*

Only secretion levels of those collagen types that were altered by *M. leprae* infection were further affected by macrophage secretory products. In SW Nfs, a decrease in collagen type IV was observed in the presence of LPS activated macrophage conditioned medium (Figure 3). In C57Bl/6 Nfs, a generalized decrease was observed in secretion levels of collagen type I on exposure to uninfected, infected or LPS activated macrophage conditioned medium



**Figure 3.** Secretory levels of collagen type IV of uninfected (□) and 3 day post-infected (■) Nf cultures exposed for 72 h to conditioned medium from uninfected (MoU), *M. leprae* infected (MoU) or LPS activated (MoLPS) macrophage culture in SW strain. Values indicated are mean ± SD of three independent experiments performed in triplicate.

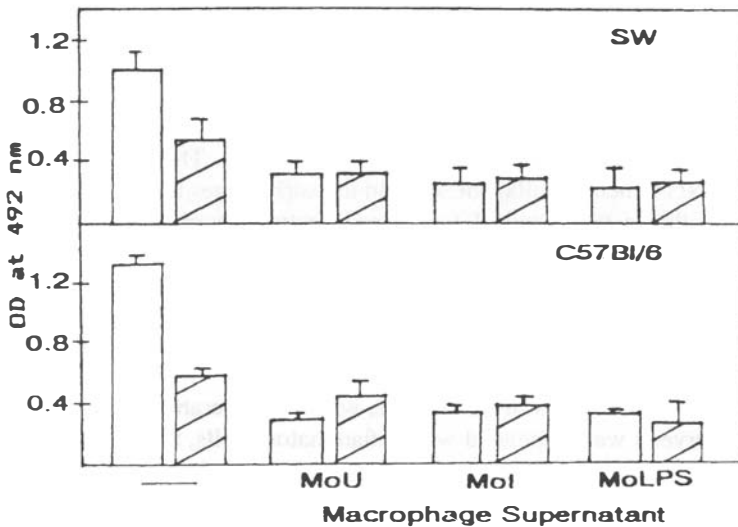


**Figure 4.** Secretory levels of collagen type I of uninfected (□) and 3 day post-infected (■) Nf cultures exposed for 72 h to conditioned medium from uninfected (MoU), *M. leprae* infected (MoU) or LPS activated (MoLPS) macrophage culture in C57Bl/6. Values indicated are mean  $\pm$  SD of three independent experiments performed in triplicate.

(Figure 4). Secretory levels of collagen type I and III by SW Nfs and types III and IV by C57Bl/6 Nfs was unaffected in the presence of macrophage conditioned medium (data not given).

#### Secretion of fibronectin

Following exposure to uninfected, *M. leprae* infected or LPS activated macrophage conditioned



**Figure 5.** Secretory levels of fibronectin of uninfected (□) and 3 day post-infected (▨) Nf cultures exposed for 72 h to conditioned medium from uninfected (MoU), *M. leprae* infected (MoU) or LPS activated (MoLPS) macrophage culture in SW and C57Bl/6 strains of mice. Values indicated are mean  $\pm$  SD of three independent experiments performed in triplicate.

medium, the levels of fibronectin secreted by both uninfected and *M. leprae* infected Nfs from the two strains were further decreased (Figure 5).

## Discussion

Fibrosis is a normal feature of wound healing. It occurs actively in tissues in response to constant trauma, or replaces areas devoid of cellular contents in a pathological condition. However, recent data implicate fibrosis as the aetiological agent in a number of conditions.<sup>13</sup> In leprosy, the presence of early signs of collagen deposition and later, a general increase in the interstitial collagen,<sup>17</sup> indicate a role for aberrant ECM deposition in the pathogenesis of leprosy nerves. Since perturbed Nf activities such as perineurial multilayering, which is an important feature of leprosy neuropathy, and increase in cell population are present, the present study was carried out to determine the contribution, if any, of Nfs in fibrosis and thus in the pathogenesis of leprosy nerve damage.

Following *M. leprae* infection the secretion of collagens and fibronectin was decreased in Nfs from both the strains. This effect was not observed on feeding cultures with heat-killed bacilli, indicating the necessity of viable bacilli and not just its physical presence for this effect. Similar studies have also shown that alteration in protein secretion level is a feature of viable *M. leprae* infected Schwann cells<sup>18</sup> and macrophages,<sup>22</sup> the other host cells of *M. leprae*.

However, in the present study, alteration in secretion of individual ECM proteins was not reflected by any change in the secretion of total proteins, which remained unaltered on infection with *M. leprae*. Fibroblasts are known actively to secrete other ECM proteins and growth factors. It has been shown that infection with *M. leprae* increases the production of nerve growth factor in mouse Nfs *in vitro*.<sup>20</sup> This discrepancy, therefore, between total protein secretion and secretion of individual ECM proteins observed in the present study might merely indicate a compensatory increase in production of other proteins. This may result in decreased secretion of some ECM proteins not being reflected as a decreased in levels of total secretory proteins.

Though collagenization is a feature of leprosy nerves across the spectrum, it is not known which cells or what other factors contribute to the fibrosis. The present study indicates a limited role for Nfs in neural collagenization in the early stages as the secretory levels of the two interstitial collagen, type I and III, following infection was either unchanged or decreased in the two strains. This is in agreement with histological observations of no Nf activity in early leprosy nerves. Substantial histological evidence<sup>17</sup> and recent *in vitro*<sup>18</sup> studies strongly indicate that in the early stages collagen deposition may be mainly by Schwann cells. It has already been demonstrated that following *M. leprae* infection, these cells secrete enhanced levels of collagen type I, III and IV.<sup>18</sup>

It is only in the later stages of the disease, when considerable neural damage has taken place and the nerve is well populated with inflammatory cells, that *M. leprae* are observed within Nfs and an increase in their population is seen. As production of the major interstitial collagen, type III, by these cells was unaffected by the actions of macrophage secretory products, this study indicates that neural collagenization in advanced leprosy nerves may be of Nf origin. Sustained production of this collagen by Nf, unaffected by macrophage secretory factors, in leprosy nerves would lead to collagenization over a period of time. This observation was, however, surprising as macrophages, in addition constitutively to

secreting several fibrogenic enzymes,<sup>26</sup> secrete a repertoire of protein degrading enzymes which includes matrix metalloproteinases that degrade interstitial collagens like collagen type I, stromelysin that acts on fibronectin and a 72 kDa collagenase that cleaves collagens like type IV.<sup>27-29</sup> In addition, studies have also demonstrated that macrophage derived secretory products themselves induce the production of proteolytic enzymes in responsive cells. The present observations on ECM production in presence of macrophage secretory products reinstates the complex regulatory mechanism that occur for ECM protein metabolism at the site of inflammatory lesions.<sup>30</sup> This is especially signified by the observation of difference in effect of conditioned medium from LPS-treated macrophages on secretion of the different collagen types by Nfs from the two strains. Conditioned medium from LPS activated macrophages would contain high levels of TNF- $\alpha$  which is suppressive in its action on ECM protein metabolism.<sup>31</sup> However, the fibrogenic cytokines present in the conditioned medium may counter this effect, resulting in unaltered levels of some of the collagens that were observed in the presence of conditioned medium in the present study.

The effect of macrophage conditioned medium on secretion of ECM proteins, especially collagens, appears to be in contrast to the response of Schwann cells. It has been shown that macrophage secretory products downregulate the secretion of ECM proteins<sup>18</sup> by Schwann cells. This supports the present observation that Nfs may be the major contributors to neural collagenization only in the later stages of nerve damage in leprosy. However, unlike the important aetiological role of collagen deposition in inflammatory conditions like pulmonary fibrosis and atherosclerosis, collagen accumulation by Nfs in the later stages of nerve damage in leprosy may be just a secondary response to inflammatory damage. The response of Nfs to inflammatory changes may simply be similar to the response of other body tissue fibroblasts to damage, with ECM proteins accumulated by them finally replacing areas devoid of cellular content in damaged leprosy nerves.

## Acknowledgements

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## Intertester reliability of manual muscle strength testing in leprosy patients

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**Summary** This study reports the results of a study on the intertester reliability of manual muscle strength testing in leprosy patients with confirmed motor function loss of at least one nerve. Three testers graded the muscle strength of 72 patients in random order. Both hands and feet were graded. Strength was graded on a modified Medical Research Council Scale (9 points, 5, 4+, 4, 3+, 3, 2+, 2, 1, 0). The following movements were tested for strength: little finger and index finger abduction, intrinsic position of all four fingers, thumb abduction and opposition, foot dorsiflexion and eversion and extension of the big toe. The weighted kappa statistic was used to calculate the chance-corrected percentage of agreement between observers. Overall agreement for each of the 11 tests appeared to be good or very good (0.61–1.00). However, when data for hands or feet with normal strength or complete paralysis were excluded from the analysis, the reliability of the remaining mid-range scale was not acceptable (kappa 0.55–0.88, direct agreement range 11–41%). While the reliability of this scale could possibly be improved by special training, we feel that, for the evaluation of nerve function for leprosy patients with (suspected) nerve function loss, the extended 9-point VMT scale should only be used when direct intra- or intertester agreement is more than 80%.

### Introduction

Manual muscle strength testing (MMST), commonly known as voluntary muscle testing (VMT) in leprosy, is an important technique in the assessment and evaluation of (motor) nerve (dys)function. Treatment decisions are often based on, and guided by the results of nerve function assessments, taking into consideration factors such as duration and severity of nerve function loss, clinical activity of the disease and findings on nerve palpation. A

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**Table 1.** Six-point scale for interobserver reliability

Medical Research Council (MRC) scale <sup>a</sup> 6 grades	Modifications 9 grades
5 Full range of motion; full resistance	4+ moderate resistance
4 Full range of motion; some resistance	3+ minimal resistance
3 Full range of motion; no resistance	2+ nearly full range
2 Decreased range of motion	
1 Muscle flicker	
0 Complete paralysis	

<sup>a</sup> The MRC scale originally grades 4–5 against gravity and 0–3 with gravity eliminated. For muscle grading of small muscles, the effect of gravity is negligible.

timely diagnosis of the disease and decreasing nerve function followed by appropriate action may prevent the development of impairments and disabilities.<sup>1,2</sup>

Muscle strength testing is used in leprosy for diagnostic purposes, in studies to assess and evaluate the efficacy of medical and surgical interventions, and in epidemiological studies. The motor nerves commonly affected in leprosy are the facial, ulnar, median, radial, common peroneal and posterior tibial nerves.

Lienhart *et al.*<sup>3</sup> reported on the interobserver reliability of MMST of one muscle test for each of the nerves that can become paralysed in leprosy. Two physiotherapy technicians graded muscle strength on a 6-point scale and two field workers on a 4-point scale. Brandsma *et al.*<sup>4</sup> assessed the intra- and interobserver reliability for some intrinsic muscles and movements of the hand in nine tests, in 27 leprosy patients with confirmed loss of ulnar or median nerves. Reliability was assessed on a 6-point scale (Table 1). Results of the above studies showed acceptable reliability coefficients. Two reasons led us to study further the reliability of manual muscle testing. First, it would be desirable to know if acceptable reliability can be maintained if the muscle strength grading scale is refined. This could have important implications for diagnostic and management purposes. Second, the reliability coefficients of the muscle strength tests are influenced by the high proportion of patients with normal or with completely paralysed muscles which are relatively easy to grade. How would reliability be affected if the normal and completely paralysed muscles were left out of the analysis? The purpose of this study, therefore, was to establish the intertester reliability of muscle strength of muscles innervated by the ulnar, median and lateral popliteal nerves on a 9-point scale in a large population of leprosy patients.

**Methods**

The study was conducted at the Green Pastures Hospital in Pokhara, Nepal. This 100-bed hospital is a referral hospital for leprosy patients in the Western Region of Nepal. For this study, both in- and out-patients were used. Informed consent was obtained from all patients. Seventy-two patients were admitted to the study.

Both hands and feet were tested. A hand or foot was not tested if there was a severe



**Table 2.** Nine-point scale used to grade muscle strength

Nerve	Movement tested
Ulnar	abduction little finger intrinsic position fingers (4) abduction index finger
Median	abduction thumb opposition thumb
Lateral popliteal	dorsiflexion foot eversion foot extension big toe

deformity or if testing was painful, which could compromise the test results. The tests have been described elsewhere by the first author.<sup>5</sup>

Intertester reliability was assessed between three testers, two experienced technicians from the hospital (5 and 10 years experience in manual muscle strength testing), and a visiting physiotherapist with more than 20 years experience in nerve function testing in leprosy patients. A 9-point scale was used to grade strength of muscles innervated by the ulnar, median and lateral popliteal nerves (Tables 1 and 2).

Prior to the start of the study, the assessors had one session to discuss the testing protocol. There was no practice session with patients prior to the study, to discover and discuss possible differences in interpretation of grades. Patients were tested in random order by the assessors, who had no information on test results of the other assessors. Testing for each patient was usually completed within 1 h.

#### STATISTICAL METHODS

Data were entered in Epi Info version 6, an integrated software program developed by the Centres for Disease Control in the United States and the World Health Organization.<sup>6</sup> Paired observer agreement was evaluated using the percentage of direct agreement (the percentage of data pairs that agreed exactly) and the weighted kappa statistic. Weighted kappa is a coefficient of agreement between observers for categorical scales of more than two categories.<sup>7</sup> It may be interpreted as the percentage of agreement between the observers corrected for chance and taking into account scaled disagreement. Quadratic disagreement weights were used.<sup>8</sup> Ninety-five percent confidence intervals (95% CI) are presented for all weighted kappa values.

Data pairs of the right and left hand (or foot) were considered statistically independent for the purpose of this study. The 72 study subjects therefore contributed up to 144 sets of three data pairs. However, data from patients for whom one of the three testers did not perform a test were discarded. Because very few patients were scored as '1', these scores were recoded as '0'. This approach is clinically acceptable, as there is very little functional difference between these grades. For analysis purposes, therefore, the remaining scale was 8-point instead of 9-point. To examine the performance of the scale in the middle range (4+ to 2), a separate analysis was done after omitting the results of hands or feet scored by all three testers as '5' (normal strength) or '0' (paralysed).

# Results

Table 3 shows the results of all the individual muscle strength tests using the data from the 9-point scale. While the weighted kappa values were mostly  $\geq 0.80$  (very good) for all three tester pairs, the direct agreement was much less good (range 40–84%). Agreement in the ‘mid-range scale’ (data from hands or feet scored ‘normal’ or ‘completely paralysed’ by all testers omitted) for six commonly tested movements is shown in Table 4. A dramatic drop is seen in both weighted kappa values and percent direct agreement (range 11–41%) for almost all sites and all tester pairs. Four typical agreement matrices are shown in Tables 5–8. Except in the upper left hand corner cells (0/0 or completely paralysed) and in the bottom right hand corner cells (5/5 or normal), very few data points lie on the diagonals. Omitting the data on both ends of the scale from the analysis, a marked reduction of the agreement indices is observed for the remaining scale. The difference between the full-scale agreement and the mid-scale agreement is illustrated in Figures 1 and 2.

**Table 3.** Intertester agreement of manual muscle strength testing (VMT) on a 9-point scale

Movement	Testers	Number of sides tested	Direct agreement (%)	Weighted kappa	95% CI
Abduction little finger	A, B	126	48	0.89	0.71–1
	A, C		44	0.86	0.68–1
	B, C		40	0.84	0.66–1
Intrinsic position index finger	A, B	120	51	0.9	0.72–1
	A, C		48	0.91	0.73–1
	B, C		52	0.88	0.70–1
Intrinsic position middle finger	A, B	123	66	0.94	0.76–1
	A, C		71	0.94	0.76–1
	B, C		66	0.94	0.75–1
Intrinsic position ring finger	A, B	121	63	0.93	0.75–1
	A, C		68	0.94	0.76–1
	B, C		63	0.91	0.73–1
Intrinsic position little finger	A, B	123	48	0.92	0.74–1
	A, C		45	0.9	0.72–1
	B, C		45	0.89	0.71–1
Abduction index finger	A, B	127	63	0.96	0.79–1
	A, C		58	0.94	0.77–1
	B, C		53	0.92	0.78–1
Abduction thumb	A, B	133	47	0.78	0.62–0.94
	A, C		56	0.82	0.66–0.98
	B, C		52	0.65	0.49–0.81
Opposition thumb	A, B	133	61	0.87	0.69–1
	A, C		59	0.9	0.72–1
	B, C		60	0.81	0.65–0.97
Dorsiflexion foot	A, B	124	78	0.94	0.77–1
	A, C		78	0.9	0.73–1
	B, C		73	0.84	0.67–1
Eversion foot	A, B	123	87	0.96	0.78–1
	A, C		84	0.87	0.69–1
	B, C		82	0.85	0.67–1
Extension big toe	A, B	112	55	0.91	0.73–1
	A, C		55	0.86	0.68–1
	B, C		46	0.81	0.63–0.99

**Table 4.** Intertester agreement of manual muscle strength testing on a 9-point scale: results after omitting hands and feet scored by all three testers as 'normal' or 'paralysed'

Movement	Testers	Number of sides tested	Direct agreement (%)	Weighted kappa	95% CI
Abduction little finger	A, B	92	30	0.76	0.56–0.96
	A, C		24	0.65	0.45–0.85
	B, C		17	0.62	0.42–0.82
Intrinsic position ring finger	A, B	66	32	0.67	0.43–0.91
	A, C		41	0.78	0.54–1
	B, C		32	0.64	0.42–0.86
Abduction index finger	A, B	72	35	0.88	0.64–1
	A, C		26	0.84	0.62–1
	B, C		17	0.77	0.55–0.99
Abduction thumb	A, B	79	11	0.49	0.30–0.68
	A, C		25	0.6	0.41–0.79
	B, C		19	0.4	0.6–0.54
Dorsiflexion foot	A, B	38	29	0.82	0.51–1
	A, C		29	0.66	0.37–0.95
	B, C		13	0.51	0.24–0.78
Eversion foot	A, B	28	39	0.88	0.49–1
	A, C		23	0.58	0.25–0.91
	B, C		15	0.55	0.22–0.88

Tables 5–7 show an asymmetrical distribution of the off-diagonal data points, indicating a systematic bias in the grading between the two testers, i.e. one tester is usually grading higher than the other tester. This bias occurred for each of the three tester pairs, only for some of the tests (data not shown). No consistent pattern could be found.

## Discussion

The results of this study highlight a methodological problem that is not uncommon in studies of repeatability of measurements using a categorical scale. Because it is often not too difficult

**Table 5.** Agreement matrix of tester B and tester C for abduction of the little finger on a 9-point VMT scale. Direct agreement 39.7%; weighted kappa 0.84. Omitting normal (5/5) and paralysed (0/0) subjects, the direct agreement is only 17.4% and weighted kappa 0.62

Category	0	1	2	2+	3	3+	4	4+	5	Total
0	30		3	2	1	1				37
1			1							1
2	1		1		1	1	1			5
2+				2	3	1			1	7
3				1		3	3		2	9
3+					4	6	21	5	3	39
4						1	2		5	8
4+						1			1	2
5							5	5	10	20
Total	31	0	5	5	9	14	32	10	22	128

**Table 6.** Agreement matrix of tester A and tester B for abduction of the thumb on a 9-point VMT scale. Direct agreement 47.4%; weighted kappa 0.78. Omitting normal (5/5) and paralysed (0/0) subjects, the direct agreement is only 11.4% and weighted kappa 0.49

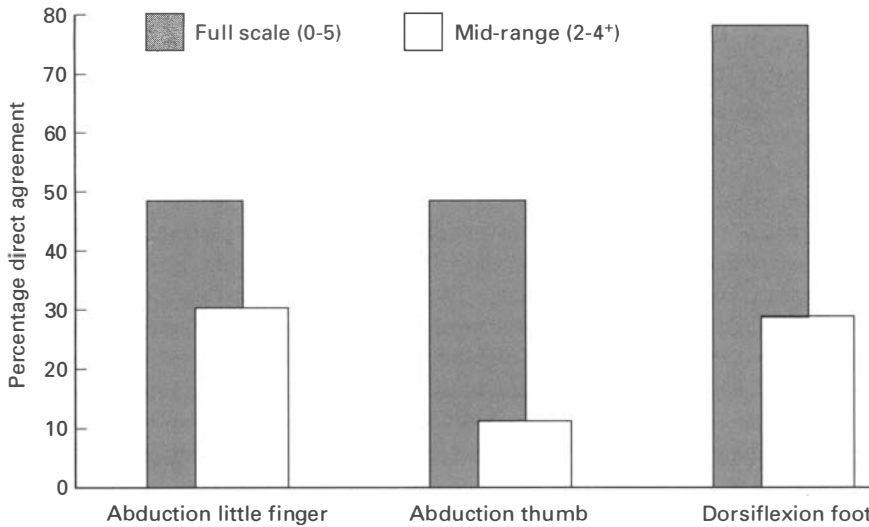
Category	0	1	2	2+	3	3+	4	4+	5	Total
0	9									9
1										0
2	1	1								2
2+	1		1	1						3
3			2	2	3	2				9
3+			2	4	3	3		2		14
4						1	1	3	4	9
4+			1		3	6	2		9	21
5				1	1	10	2	6	46	66
Total	11	1	6	8	10	22	5	11	59	133

**Table 7.** Agreement matrix of tester B and tester C for dorsiflexion of the foot on a 9-point VMT scale. Direct agreement 73.4%; weighted kappa 0.84. Omitting normal (5/5) and paralysed (0/0) subjects, the direct agreement is only 13.2% and weighted kappa 0.51

Category	0	1	2	2+	3	3+	4	4+	5	Total
0	6									6
1			1			1				2
2						1				1
2+			1			1	3			5
3							1			1
3+							1	1	4	6
4										0
4+							1	1	9	11
5						3		4	84	91
Total	6	0	2	0	0	7	6	6	97	124

**Table 8.** Agreement matrix of tester A and tester B for abduction of the index finger on a 9-point VMT scale. Direct agreement 48.4%; weighted kappa 0.89. Omitting normal (5/5) and paralysed (0/0) subjects, the direct agreement is 30.4% and weighted kappa 0.76

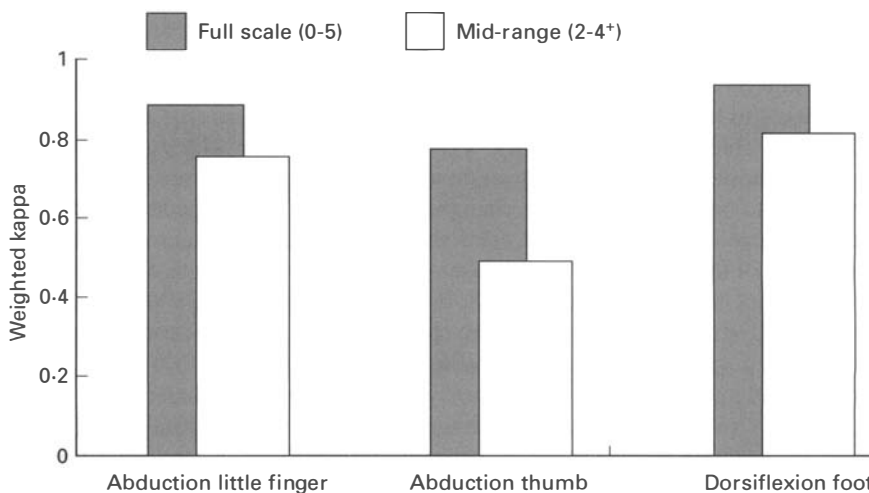
Category	0	1	2	2+	3	3+	4	4+	5	Total
0	22									23
1		1								1
2	2		2	1						5
2+		1	1	2	1	1				6
3			1	2	2	3				8
3+						3	1			4
4						6	0	2	2	10
4+							7	4	9	20
5						2	2	3	44	51
Total	24	2	4	5	3	15	10	9	55	127



**Figure 1.** Differences between direct intertester agreement on the full 9-point VMT scale and on the 'mid-range' scale (hands and feet scoring normal or completely paralysed omitted).

to tell whether a test is completely normal or completely abnormal, agreement between testers tends to be good on both extremes of the scale. If there are many normal and completely abnormal test results, these will have a disproportionate effect on the agreement indicators that are calculated.

Between-tester agreement using the full 9-point scale appears good (Table 3), although the relatively low direct agreement is striking compared to the high values of the weighted kappas. No absolute standard exists for assessing kappa values, but many investigators use



**Figure 2.** Differences between intertester agreement (weighted kappa) on the full 9-point VMT scale and on the 'mid-range' scale (hands and feet scoring normal or completely paralysed omitted).

the classification suggested by Altman: kappa 0.61–0.80: good; kappa 0.81–1.00: very good.<sup>8</sup> Using this classification, agreement between testers appears to be very good for most movements tested. Taken at face value, one would conclude that the 9-point VMT scale has very good reliability and can therefore be recommended for clinical use.

However, the clinical use of a rating scale such as the VMT is particularly important for patients who have some nerve dysfunction. Therefore, the performance of the scale in the mid-range, i.e. when there is muscle weakness, is at least as important as its ability to rate nerves as 'normal' or 'paralysed'. To investigate the mid-range performance of the 9-point VMT scale, we reanalysed the results omitting the data from muscle (groups) that were rated normal or paralysed by all three testers. The conclusion from this analysis (Table 4) is different. Direct agreement was very low, while the kappa values also dropped considerably. It should be noted that the value of weighted kappa is dependent on the prevalence of the individual grades and the number of categories of the scale. A decrease in the value of kappa after trimming the ends of the scale is therefore not unexpected. However, the size of the decrease and its concurrence with a marked decrease in direct agreement show that this is not due to an artifact.

The sample of agreement matrices shown in Tables 5–8 reveals a wide scatter of data points, with often more than one category difference between the testers. An exception is the rating of abduction of the index finger (Table 8), where the scatter is much less. Nevertheless, the mid-range direct agreement for this test was only 17.4–30.4%, depending on the tester pair. There was evidence of systematic bias between the testers at least for some of the tests. This could not be traced back to one particular tester or one or more specific tests. It is likely that specific training addressing these differences would result in improved reliability.

The benefit of the 9-point scale over the conventionally used 6-point scale is that it has more categories in the mid-range and is therefore potentially more sensitive than shorter scales. However, the current study shows that the rating of muscle strength in this mid-range is particularly unreliable. We conclude that the use of such an extended VMT scale should not be recommended, unless perhaps after special training reliability (direct inter- or intratester agreement) is more than 80%. It should be noted that the current results refer to inter-tester repeatability. It is likely that intra-tester repeatability would be better, but this should be investigated in a separate study.

A disadvantage of manual testing is that muscle strength is evaluated against what the examiner deems to be normal for a particular patient. This means that much experience is required for reliable testing, especially when children or the elderly are involved. To overcome this problem, the use of dynamometers has been recommended. Dynamometry has been shown to be more sensitive to changes in the MRC 3–5 range than MMST.<sup>9–12</sup> The grip and pinch dynamometers for the hand show excellent instrument reliability.<sup>13,14</sup> The additional value of dynamometers in the assessment of muscle strength of the hand and foot in leprosy patients needs to be investigated. In the 3–5 range on the VMT scale, the values obtained with a dynamometer could confirm changes that are also noticed with the VMT, or could indicate changes that are not noticeable on manual testing. VMT and dynamometry could be assessed and recorded simultaneously, as suggested by Brandsma.<sup>15</sup>

It is important to realize that in strength testing in the so-called 'lumbrical position', the interosseus muscles are tested rather than the lumbricals. This is especially important for the index and middle fingers, which, in more than 95% of patients with an ulnar palsy, will be weak or may even 'claw'.<sup>16</sup>

At the outset of the study, it was not planned to test big toe extension (extensor hallucis

longus, EHL). Big toe extension, however, is routinely assessed at the Green Pastures Hospital and the two physiotherapists who participated in the study indicated that they often recorded isolated weakness of big toe extension, with normal strength of eversion and dorsiflexion. It was therefore decided to include this test in the study. Isolated weakness of EHL was observed in 26 feet (22 patients), which is 23% of all feet tested. Is it possible to have isolated damage of the nerve branch supplying the EHL? Could it be that weakness in the EHL precedes weakness in dorsiflexion and eversion? An answer to these questions needs to be obtained through a prospective study.

Fritsch, Jehin and Palande recommend the use of so-called functional muscle tests in the field, e.g. thumb to little finger opposition.<sup>17-19</sup> It should be realized that these tests may still be negative when 50-70% of muscle strength has already been lost.<sup>20</sup> They are only of (limited) value when patients or suspected patients have to be screened in large numbers or when competency and quality of the leprosy staff does not allow for grading of muscle strength.

In conclusion, in this study, the mid-scale range (4+ to 2) of the 9-point VMT scale showed unacceptable intertester reliability. Therefore, we recommend that the 9-point scale (or any further sophistication, such as an 11-point scale) in the assessment of nerve function in leprosy patients is only used when acceptable direct intra- or intertester agreement has been obtained.

Caution is needed when basing conclusions of reliability studies on weighted kappa values alone. This is particularly true if the study population contains a high proportion of normal or completely abnormal test results. Direct agreement, reliability of the mid-scale range and examination of agreement matrices can give essential extra information.

The use of dynamometry as an adjunct to manual muscle testing in leprosy should be investigated.

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## **Leprosy and disability control in the Guéra Prefecture of Chad, Africa: do women have access to leprosy control services?**

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*Summary* In a retrospective study, data from the Guéra Leprosy and Disability Control Project in Chad, covering the years from 1992 to 1996, were analysed in order to determine whether there was any indication that the quality of care provided to female leprosy sufferers is inferior to the care provided for male patients. Data from a total of 741 patient registered for MDT, of whom 351 were newly diagnosed cases, are presented and discussed. The data indicate that women have access to diagnosis and treatment and health education. They do not present for treatment later than men, disability rates are lower and they have slightly higher treatment completion rates. Both women and men benefit from footwear and loan programs. More women than men are involved in patient self-help groups. The study shows that in this part of central Chad, there is no evidence of disadvantage for women with leprosy in either diagnosis, treatment or follow-up, but more qualitative data is needed to confirm these findings.

### **Introduction**

Systematic discrimination against women has been recognized in many cultures world-wide. While some aspects of discrimination are quite obvious even to the casual observer, more subtle and complex mechanisms exist that are much more difficult to identify. There is an increasing awareness that such discrimination may affect activities that are primarily considered to be gender neutral. Attention has been drawn to the relationship of gender and health and the significant disparities that exist between women and men,<sup>1</sup> and recent research on gender and tropical disease has highlighted some of the particular disadvantages that women face with respect to risk factors of infection, the social costs of disease and access to care.<sup>2–4</sup>

Within the framework of leprosy control activities, two distinct phases can be identified during which discrimination against women could occur. The first phase of case detection and diagnosis covers the time between the onset of the disease and ends when the diagnosis of leprosy is established and the patient is registered for treatment. The key question during this phase is whether facilities for diagnosis and treatment for leprosy are readily accessible to members of both sexes, or whether there are factors that prevent women from presenting for

diagnosis and treatment. It has been pointed out that in India, because of fear of social ostracism, women are more likely to hide their disease than men<sup>5</sup> and that significantly fewer females were detected through voluntary reporting compared to active case detection in the community.<sup>6</sup> Awofeso notes that in northern Nigeria, female and male patients differ in significant respects in their perception of socio-cultural beliefs about leprosy and discusses the possible implications for health education programmes.<sup>7</sup> Many of the factors that affect case detection are beyond the direct influence of the leprosy control service, but their recognition and analysis are key elements to eventual improvement.

The second phase is that of treatment and follow-up. The key question here concerns the quality of treatment and care: is it provided regardless of the sex of the patient once the diagnosis has been established, or does overt or hidden discrimination take place, resulting in an inferior quality of care for female leprosy sufferers? A wide variety of mechanisms through which discrimination can occur are conceivable, particularly in a male dominated health care system. As much of this discrimination originates within the service itself, it can be addressed and corrective action can be taken.

In a recent policy statement on gender, The Leprosy Mission International (TLMI) affirms its commitment to 'maximize women's access to leprosy diagnosis, health education, health care and rehabilitation'.<sup>8</sup> The implementation of such a policy presents a particular challenge to projects working in cultural environments where male dominance in all aspects of public life is an established and unquestionable fact.

This is the case for the Guéra Leprosy and Disability Control Programme, a TLMI project situated in the Guéra Prefecture in central Chad and started in 1992. It has taken an active role in implementing multi-drug therapy (MDT) within the framework of the National Leprosy Control Programme of Chad (NLCP).

There is an extremely high illiteracy rate of 94·8% among women, compared to 77% among men and the low school attendance rate for girls. In rural areas, only 15% of the girls attend primary school, while the rate for boys is 27%.<sup>9</sup> Only a small minority of the health care staff are women. Apart from two expatriate workers in our project (POD officer and leprosy supervisor), all NLCP staff at national and provincial level are men.

In the following, I will review some of the activities undertaken by the Guéra Leprosy and Disability Control Project from 1992 to 1996 in order to determine whether the available data provide any indication that quality of care provided to female leprosy sufferers is inferior to the care provided for male patients.

## **Materials and methods**

### **BACKGROUND INFORMATION ON THE GUÉRA PREFECTURE**

The Guéra Prefecture, located in central Chad, has a population of 335,000 scattered over an area of 60,000 km<sup>2</sup>. Women outnumber men by a ratio of 1·17:1; this is higher than the national female/male ratio, which lies at 1·06:1.<sup>10</sup> Approximately two-thirds of the population belong to the so called 'Hadjerai' (people of the rock). While this is not a homogeneous group, but is composed of a number of small ethnic groups that speak distinct languages, these groups share many cultural traits. They live as sedentary subsistence farmers, whose main crops are millet, ground nuts and sesame. About 15% of the population are nomads of predominantly Semitic origin, who raise cattle, goats and camels. The predominant religion is Islam: 90% of the population are Muslims, 5% are animists and

Christians make up 3%. At least 15 different languages are spoken, chadian Arabic is used as the trade language. There are no surfaced roads and the road network is very poor. Access to many areas is difficult during the dry season and impossible during the rainy season.

At the peripheral level, health care is provided by health centres that serve a defined health zone. However, only 12 out of 32 designated health zones actually have a health centre. The health zones are grouped into three districts, which are theoretically served by a district hospital. However, only one hospital exists for the entire prefecture, and district medical officers were only posted in 1996. Even where health facilities exist, quality of health care is often poor due to the lack of qualified staff and material resources.

Leprosy treatment is provided free of charge as one of the services provided by the health centres.

Data provided by the NLCP for all of Chad shows that in December 1995 the prevalence of leprosy was estimated at 4.91 cases per 10,000, with 3238 leprosy patients on register; of these 3104 (96%) were on MDT. The case detection rate for 1995 was 15.4 per 10,000, with 1015 new cases detected in the course of the year.<sup>11</sup>

#### GUÉRA LEPROSY AND DISABILITY CONTROL PROJECT

Multi-drug therapy was introduced in the Guéra in 1992 after leprosy work had been disrupted for more than 10 years as a result of the long civil war. A phased approach was adopted for MDT implementation.

In a first phase of MDT implementation, a review of all known cases was undertaken and no particular case finding efforts were made. A cycle of supervision was established, health centre staff was trained and a technical manual<sup>12</sup> was written that was later adopted by the national leprosy control programme. By June 1994, MDT had been introduced in all health centres.

The second phase was characterized by the transfer of responsibility for treatment to health centre staff and an intensification of case finding through health education of the general public, and continued regular monthly visits by the leprosy supervisor. The third phase of extension of MDT into areas not covered by health services began after MDT was well established in all health centres. The approach used and the results obtained has been described elsewhere.<sup>13</sup> By the end of 1996, patients had been put on MDT in all but three of the 32 designated health zones.

#### DIAGNOSIS, CLASSIFICATION AND TREATMENT

Responsibility for diagnosis, classification and treatment lay with the leprosy supervisor. The leprosy supervisor in turn is supervised by the medical officer. As a rule, all patients were seen at least once by the medical officer.

Diagnosis, classification and treatment followed the procedures outlined in the Technical Manual. Briefly, diagnosis was based on the presence of at least one of three cardinal signs (anaesthetic lesion, nerve enlargement or the presence of acid fast bacilli in slit skin smears). From mid-1995 onward, all patients with more than five skin lesions were classified as multibacillary (MB), regardless of their bacillary index (BI). Only cases that had no history of previous treatment with anti-leprosy drugs were considered to be new cases. All other cases were regarded as 'old cases', regardless of the duration of previous treatment. Patients were treated using standard WHO-MDT blister packs. Treatment was discontinued after 6 months

for paucibacillary (PB) cases and after 24 months for MB cases. PB patients who were absent for 3 consecutive months or who could not complete 6 monthly doses in 9 months were considered 'lost to follow-up' (LF). MB patients who could not receive 24 monthly doses within 36 months or who were absent for 6 consecutive months were also considered LF. LF cases were removed from the treatment register and readmitted for another full course of treatment under category 'Readmitted' if they presented at a later stage with signs of active disease.

The clinical examination at the time of diagnosis included recordings of all visible deformity and a nerve function assessment with voluntary muscle testing and sensory testing using graded nylon filaments for all patients. Deformities of eyes, feet and hands were recorded for all patients using the WHO 2 point disability grade,<sup>14</sup> and the highest of these was also recorded as the overall disability grade of the patient.

#### PREVENTION OF DISABILITY

From the outset, special attention was given to prevention of disability (POD) activities as an integral part of MDT implementation. This included regular testing for changes in muscle strength of eyelids, hands and feet by a standard set of voluntary muscle tests (VMT) and changes in the sensitivity of palms and soles by sensory testing (ST) with graded nylon filaments for all patients on MDT, health education for patients with existing neurological deficits, a protocol for field treatment of neuritis, provision of locally produced protective footwear for patients with plantar sensory loss and ulcer care.

Two main problems were identified:

The patients were scattered over a vast area and therefore only seen once a month by the leprosy supervisor. Given the high workload, POD activities were often unsystematic and follow-up was poor. Second, patient education required extra time and special skills that the understaffed health centres could not provide. In order to deal with these problems, four health educators were recruited and trained as POD assistants and assigned to certain geographical areas. All four originated from their respective work zones. In training, special emphasis was placed on communication skills.

A task oriented POD Manual<sup>15</sup> was written, describing in detail all activities and procedures that were considered necessary and practicable, and evaluation parameters were defined. This included a protocol for standardized health education for all patients registered for MDT. At the time of diagnosis, patients received a short education session with four simple messages, and the fact that education had been given was recorded on the patient record. On a subsequent visit to the clinic, comprehension of the health education previously received was evaluated by a set of standardized questions. If any of the responses was incorrect, the health education message was repeated.

#### METHODS AND STATISTICAL ANALYSIS

All patient records were specifically designed to facilitate data collection. A computerized relational database containing individual patient data was set up in 1993 using EPI-Info 5. Individual patient data were collected as part of routine activities by the leprosy supervisor when the patient registered for treatment and entered into the database on a quarterly basis. Printouts of data were produced in quarterly intervals and verified during supervisory visits by the medical officer. The data collected included general data such as age, sex, distance

from treatment centre, lifestyle (sedentary or nomadic), and leprosy-related data such as date of diagnosis and start of treatment, time elapsed between first symptoms and diagnosis, history of previous treatment, Bacterial Index, WHO classification, and disability grades for hands, feet and eyes.

With the start of the POD programme in 1994, additional information on health education, supply of footwear and appliances was routinely collected by the POD officer and entered into the database.

Data were compiled from the existing database. Statistical analysis was carried out by the  $\chi^2$  test using the Epi Info Version 6 package.<sup>16</sup> The level of significance was set at 95% ( $p < 0.05$ ).

## Results

### PATIENTS REGISTERED FOR MDT (FIGURE 1)

Of 351 patients put on MDT from 1992–1996, 206 (59%) were women or girls with variations in the yearly rate ranging from 48% (1994) to 64% (1996). The variation over the years was not statistically significant.

Of the same 351 patients 29 were younger than 15 years at the time of diagnosis, 17 (58%) of them were girls.

Of 390 readmitted patients put on MDT from 1992 to 1996, 229 (59%) were women, with the variation ranging from 57 to 61% (data not shown).

### MB/PB RATIOS AMONG NEW CASES (FIGURE 2)

Among the new patients, 108 (31%) were classified as having multi-bacillary (MB) leprosy. There was a statistically significant difference between men and women, with an MB rate of 26% among female patients compared to 38% among male patients.

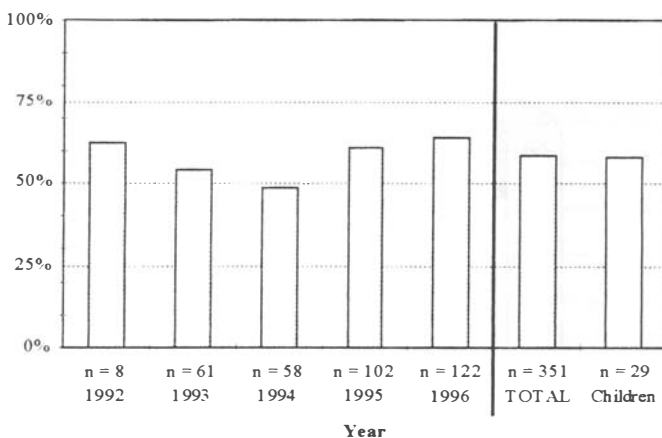


Figure 1. Percentage of women among newly diagnosed leprosy patients, 1992–1996.

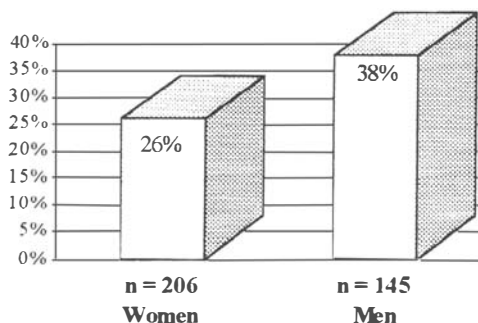


Figure 2. Multibacillary rate among newly diagnosed cases, by gender, 1992–1996.

#### DISABILITY GRADE AT TIME OF DIAGNOSIS (FIGURE 3)

A statistically significant lower rate of disability was found among newly diagnosed female patients. Among women, 58% had no disability or deformity at time of diagnosis (WHO disability grade 0), compared to 43% among new male patients. This difference was more marked among PB cases, where 65% of women and only 47% of men had no disability or deformity. Among MB cases, the difference in disability rates were statistically not significant, only 40% of women and 36% of men had a disability grade of 0 at the time of diagnosis.

#### TIME ELAPSED BETWEEN FIRST SYMPTOMS AND DIAGNOSIS (FIGURE 4)

At the time of diagnosis, all patients were asked how long ago they had noticed the first signs of the disease. Only 13% of women and 11% of men had noticed the first signs less than a year ago, while 44% of women and 43% of men said that they had been ill for more than 5 years already.

Of the new patients only 12% had noticed the first signs less than a year ago, while 43% had been ill for more than 5 years. The small difference observed between female and male patients was not significant.

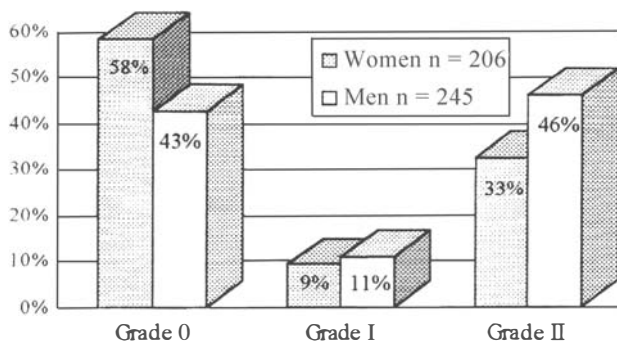


Figure 3. WHO Disability Grade I in newly diagnosed cases, 1992–1996.

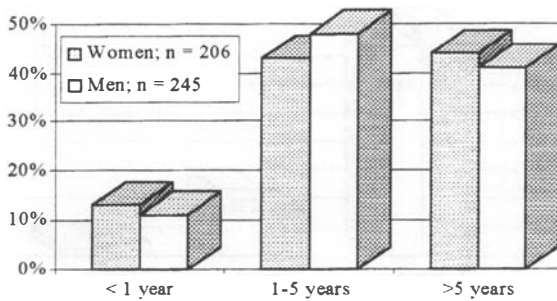


Figure 4. Duration of disease before diagnosis in newly diagnosed leprosy patients, 1992–1996.

#### TREATMENT COMPLETION RATES, MB CASES (FIGURE 5) AND PB CASES (FIGURE 6)

Treatment compliance can only be studied for those patients that have had the chance to complete their treatment within the defined treatment period, i.e. 36 months for MB cases and 9 months for PB cases. Thus only MB cases that started treatment before 1995 and PB cases that started before 1996 are considered. Of 202 MB cases put on treatment between 1992–1994, 144 (71%) received 24 monthly doses of MDT within 36 months. Thirty-eight patients (17%) were lost to follow-up, either because of more than 6 consecutive months of absence or for more than 12 months cumulated absence from treatment. There were no differences in treatment completion rates and the defaulter rates between men and women. Among the 335 PB cases put on treatment between 1992–1995, treatment completion rates for women were significantly better than treatment completion rates for men (79 versus 74%), with higher defaulter rates among male patients.

#### HEALTH EDUCATION UPON DIAGNOSIS (FIGURE 7)

Health education using a standardized format was started in 1994. Out of the 260 patients for whom data are available, 90% of women and 96% of men registered for MDT received health education at the time of diagnosis; the difference between the sexes was not significant. Only 21% of women and 23% of men were able to answer all questions correctly on the subsequent visit.

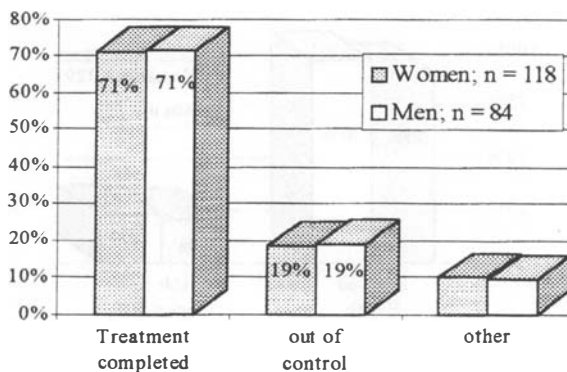


Figure 5. Treatment outcome in multibacillary cases, by gender, 1992–1996.

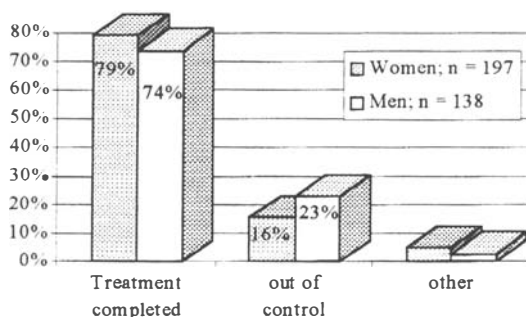


Figure 6. Treatment outcome in paucibacillary cases, by gender, 1992–1996.

#### PROVISION OF FOOTWEAR TO PATIENTS WITH INSENSITIVE FEET (FIGURE 8)

Locally produced protective footwear was offered to all patients with plantar sensory loss at a nominal price. Out of 370 patients with plantar sensory loss, 167 (45%) have received at least one pair of MCR sandals. The difference observed between women and men (44 compared to 47%) was not significant.

## Discussion

The overall epidemiological situation regarding sex distribution in leprosy is far from clear. Although leprosy affects both sexes, in most parts of the world, males are affected more frequently than females, often in the ratio of 2:1, with the difference being more marked among adults; however, male preponderance is not universal, particularly in Africa.<sup>17</sup>

In Ethiopia, among 286 new cases registered for MDT within the AMFES project from 1988 to 1992, only 37% of the patients were women (47% of the PB cases and 28% of the MB cases)<sup>18</sup> and of 5131 patients registered for MDT in the Shoa Administrative Region between 1984–1988, 35.1% of the patients were female.<sup>19</sup> In a limited study conducted in the Sudan in a rural setting that resembles the situation encountered in Chad, 44% of the 43 cases detected were women.<sup>20</sup> At the Leprology Unit of the Institut Marchoux in Bamako, which serves as a

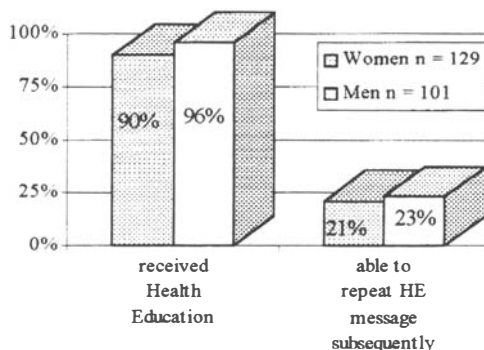
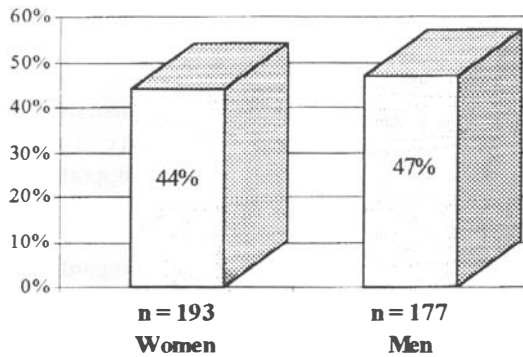


Figure 7. Initial health education.





**Figure 8.** Percentage of patients with insensitive feet who received protective footwear, by gender, 1992–1996.

reference centre for Mali, out of 187 new cases detected between February 1992 and June 1994, only 56 (30%) were women.<sup>21</sup> The authors note that the sample is not representative of leprosy patients diagnosed in the field, but no further details are given. By contrast, data from Karonga district in Malawi, based on total population surveys, show an overall higher incidence of leprosy among women than men, 61% of all the 489 cases registered were women. However, among the small group of 29 MB cases in this study, only 45% were women.<sup>22</sup>

Our own data show a clear preponderance of women among the new cases detected, not only young adults but also among children. This has been a consistent finding over the years, and applies not only to new cases but also to patients previously registered for monotherapy.

In comparing the data, it must be borne in mind that case detection rates depend on a number of factors, specifically the way case finding is organized and conducted and the fact that these rates reflect incidence and prevalence only indirectly. However, it is most probably safe to say that if ascertainment bias occurs, it would lead to under-detection of leprosy among females because women generally have less access to health care facilities and because one would expect examination of women to be less complete and less satisfactory in some cultural contexts.

The fact that in our programme more women than men are registered for MDT can be interpreted in a number of different ways. It is possible that these figures reflect an epidemiological situation similar to that encountered in Malawi, where there was higher incidence among women than among men.

It is also possible that the difference is not due to higher incidence but rather to other factors. In conjunction with the significantly lower disability rates observed in women with PB leprosy, it could indicate that women seek treatment earlier than men. However, lower disability rates among female patients have been observed in a variety of different settings such as India,<sup>23,24</sup> China,<sup>25</sup> Malawi<sup>26</sup> and Burkina Faso.<sup>27</sup> One explanation given for this finding is that women's chances of developing deformities are reduced due to less frequent involvement of nerve trunks.<sup>1</sup> When questioned about the duration of disease before diagnosis, no difference between the sexes could be elicited. By contrast, a study from Kuwait showed that women clearly had a longer time lag between onset of symptoms and presentation to the physician.<sup>28</sup> It is possible that the time scale used is too crude to detect subtle differences.

Factors leading to a lower prevalence among men, such as higher mortality rates among men or a higher rate of migration among men, could also account for higher case detection rates among females.

However, the fact that we observed higher MB rates in men than in women, a finding that is in keeping with the observation that male preponderance is much more pronounced in lepromatous leprosy than in tuberculoid leprosy, does not support this hypothesis, as there is no apparent reason why higher mortality or the migration of men should only affect PB cases.

As our data are based on passive case detection and no data from population surveys are available, it is not possible to make a conclusive statement regarding sex specific prevalence rates, although our findings suggest that more women than men are affected in our area. Whatever the case may be, the fact that we have consistently registered more female than male leprosy cases seems to be an indication that the leprosy control services are readily accessible to women.

The second question that needs to be addressed concerns the quality of care provided to female leprosy patients once the diagnosis has been established. Treatment completion rates can be used as an indirect indicator for the quality of care provided.

Regular monthly visits to the health centre over a longer period are much more likely if a good relationship has been established with the patient and if the need for regular long term treatment has been clearly understood. Treatment completion rates in our program lie between 70 and 80%, a rate that we consider acceptable under the prevailing conditions. Because many of the patients live far away from the treatment centres and access by road is difficult, defaulters cannot always be traced. The fact that women have slightly higher treatment completion rates is reassuring. Health education was given both to men and women, and seemingly there was no difference in comprehension. The fact that less than a quarter of the patients could repeat all four health education messages after a month indicates that the quality of health education needs to be improved. Because of the wide variety of languages spoken, the education can often only be done in Arabic. The fact that hardly any of our patients have had any schooling also plays an important role, the concept of 'the right answer' is often not understood. On this background, it is surprising that there are no larger differences between men and women, as men usually have a much better command of Arabic and are more likely to have had schooling.

The fact that there is no sex difference in the provision of protective footwear is interesting, particularly considering that patients are asked to pay a small fee in order to receive shoes. In a survey conducted among 246 healthy villagers in the area, only 24% of the women wore shoes compared to 60% among the men. The explanation given was that women often do not have shoes because they are considered too expensive. This does not seem to apply for protective footwear; it seems to be considered useful enough to warrant the expenditure.

The question of how effective footwear is in preventing episodes of ulceration has been addressed in a separate study (own published data), preliminary analysis of data shows no significant difference between the sexes regarding the occurrence of ulceration and bone loss.

## **Conclusion**

The data available does not provide any indication that there is discrimination of women within the Guéra Leprosy and Disability Control Project. Women have access to diagnosis

and treatment and health education. They do not present for treatment later than men, disability rates are lower and they have slightly higher treatment completion rates. Both women and men benefit from footwear. However, a study of the social consequences of leprosy may lead to different conclusions.<sup>4</sup> Among our patients, we have observed that divorce rates among female leprosy patients are much higher than among men, this has also been documented from other parts of the world.<sup>7,34</sup> Therefore, it is very likely that women suffer considerably more from the social consequences of the stigma attached to leprosy than men. As this study was not designed to address this issue, this information has not been included. Considering the overall context of this project, the findings with regard to the availability and quality of services are both surprising and reassuring; in this part of central Chad, there is no evidence of disadvantage for women with leprosy in either diagnosis, treatment or follow-up, but more qualitative data are needed to confirm these findings.

## Acknowledgements

The Guéra Leprosy and Disability Control Project is funded by The Leprosy Mission International (TLMI). The author would particularly like to thank Dr Fatchou Gakaitangou and Dr Bambé Lamtoin for their continuous support, advice and encouragement. Marjan de Koning, Yacoub Adoum and Geeske Zijp have made outstanding contributions to the field work. Dr A. C. McDougall's advice in preparing the manuscript has been most valuable.

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## Use of a whole blood assay to monitor the immune response to mycobacterial antigens in leprosy patients: a predictor for type 1 reaction onset?

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**Summary** Longitudinal studies are more appropriate than cross-sectional studies for investigating changes in the immune response to *Mycobacterium leprae* during leprosy, such as occur in type 1 (reversal) reactions. A test for predicting the onset of reactions in leprosy would greatly reduce disability associated with leprosy. Whole blood assays are appropriate for longitudinal studies of the *in vitro* T-cell response, as they are robust and reproducible, and require only a small volume of blood. Whole blood assays were used to assess the natural variation in the 'normal' T-cell response to mycobacterial antigens in healthy UK donors, and healthy Nepali donors, tested over 6 months. This was compared with variation in T-cell responses measured over 6 months in 22 leprosy patients in Nepal, including eight who developed type 1 reactions during this time. The *in vitro* T-cell response to *M. leprae* sonicate, *M. tuberculosis* PPD, the mitogen PHA, and (in the UK study) recombinant mycobacterial antigens (70 kD and 30/31 kD proteins) was measured by lymphoproliferation and interferon-gamma (IFN $\gamma$ ) responses, and variation in responses over time in each subject calculated as a coefficient of variation (CV). The baseline high, low or non-responder status of the healthy UK donors remained stable. The magnitude of IFN $\gamma$  responses varied by mean CV ranging from 26% (to PPD) to 63% (to Mtb 70 kD); proliferation responses showed less variation, ranging from mean CV of 18% (to PHA) to 47% (to Mtb 70 kD). Response variation was independent of lymphocyte number in culture. Similar variation in lymphoproliferation responses to MLS, PPD and PHA was observed in the group of healthy Nepali subjects, and in Nepali leprosy patients who did not experience reactions during the study. Of the eight leprosy patients who developed type 1 reactions, four (two BT, one BB, one BL) showed significantly increased proliferation to MLS at the time of reaction (74–300% above baseline); four (one BB, two BL, one LL) remained low or non-responders to MLS throughout. An alternative marker of immune response—anti-phenolic glycolipid-1 (PGL-1) antibody titre—was not predictive of reaction onset in these patients. This study demonstrated that whole blood assays provide

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reproducible *in vitro* measurements that can be used to monitor changes in T-cell responses to *M. leprae* antigens; their practical use as a diagnostic marker of type 1 reaction onset is discussed.

## Introduction

Studies of the human *in vitro* T-cell mediated immune response to *M. leprae* and constituent antigens are usually cross-sectional, with subjects tested at a single time point. It is becoming increasingly clear that the cell-mediated immune response is dynamic, exhibiting diurnal rhythmicity,<sup>1</sup> and influenced by hormonal changes<sup>2,3</sup> and concurrent infections.<sup>4</sup> Thus, it is important to be able to estimate the natural variability of *in vitro* T-cell responses, in order to evaluate the significance of responses measured in cross-sectional studies. A limitation to longitudinal analysis of T-cell mediated responses in sufficiently large study groups has been the large volume of blood required from the subject for each test. This problem has now been overcome with the development of whole blood assays, which can analyse lymphocyte proliferative and cytokine responses to antigens in a very small volume of blood. Whole blood assays have been used in longitudinal analyses of cellular responses in different infectious diseases, such as HIV<sup>5</sup>, leishmaniasis<sup>6</sup> and meningitis.<sup>7</sup> A whole blood assay has also been developed to analyse *in vitro* T-cell responses to *M. leprae* antigens.<sup>8</sup>

The symptoms of leprosy, including skin lesions and neuritis, are manifestations of the host immune response to infection with *M. leprae*.<sup>9</sup> Shifts in the immune response are most clearly indicated by the clinical symptoms of type 1 'reversal' reactions, frequently associated with borderline (BT/BB) leprosy, and type 2 erythema nodosum leprosum (ENL) reactions, associated with borderline lepromatous and lepromatous (BL/LL) leprosy. Reactions are the principal cause of disability in leprosy patients, as nerves local to the reaction can become irreversibly damaged.<sup>10,11</sup> The exact factors that precipitate onset of reactions have not yet been defined, although a sudden increase in *M. leprae* antigens released as a result of drug-induced bacterial killing may be a major factor. The first 6–12 months of multi-drug therapy (MDT) is considered to be the period of highest risk for the development of type 1 reactions. Both type 1 and type 2 reactions can be controlled with the steroid prednisone, indicating that an increase of pro-inflammatory activity against *M. leprae*, including release of the cytokine interferon-gamma (IFN $\gamma$ ), may be involved in the onset of reactions.<sup>12,13</sup> However, steroid treatment often starts long after the initiation of the inflammatory process, by which time nerve damage has occurred.

A means of predicting the onset of reactions would greatly reduce the incidence of disability in leprosy. Longitudinal studies of leprosy patients undergoing reactions have found increased levels of circulating soluble IL2 receptor<sup>14–16</sup> and  $\beta$ 2-microglobulin<sup>17</sup> in both types of reaction, and increased serum IgG1<sup>18</sup> and TNF $\alpha$ <sup>19</sup> in type 2 reactions, as evidence of the increased immune response underlying reactions. Lepromin positivity (indicating T-cell responsiveness to *M. leprae*), and a positive skin smear, and seropositivity for anti-PGL-1 antibodies (indicating a high antigen load which may predominate in the nerves), have been found to be associated with the tendency of leprosy patients to develop type 1 reactions.<sup>20, 21</sup> As an alteration in the T-cell response to *M. leprae* is likely to be the primary event in a reaction, monitoring of the *in vitro* T-cell response could provide an appropriate indicator of reaction onset. Previous studies have carried out lymphocyte transformation tests to measure the response to *M. leprae* in patients before, during and

after type 1 reaction, and have reported increased responses during reactions.<sup>22,23</sup> However, these studies did not attempt to define the normal variability of the human T-cell response to *M. leprae*, and some post-reaction responses were measured while the patient was still receiving immunosuppressive steroid treatment.

Therefore a more thorough assessment of the natural variability of the *M. leprae*-specific T-cell response, and the implications of changes in this for development of type 1 reaction onset, is required. Using the whole blood assay, a longitudinal study of healthy subjects in the UK was performed, to analyse the variability of T-cell responses measured every month for 6 months to *M. leprae* antigen [*M. leprae* sonicate (MLS)] in these subjects. Responses were also measured to *M. tuberculosis* purified protein derivative (PPD), and defined recombinant proteins derived from *M. leprae* (30–32 kD) and *M. tuberculosis* (70 kD)—these antigens are shared with other mycobacterial species and are therefore likely to be recognised by UK donors. Response variation in the UK subjects was compared with that measured (using identical protocols) in a group of healthy control subjects in a leprosy-endemic region of Nepal, to investigate whether increased exposure to *M. leprae* and *M. tuberculosis* in Nepal might affect the variability of responses. These baseline data were used to assess variation of T-cell responses to MLS and PPD in a group of leprosy patients in Nepal, some of whom experienced type 1 reactions during the course of the study. Variation in sequential anti-PGL-1 antibody responses in the patients was also assessed, as an additional marker of immune response change.

## Materials and methods

### SUBJECTS

In the UK, six laboratory personnel [all Caucasian; three male, three female; mean age 29 (range 25–40)] were recruited. All were BCG vaccinated; none came into contact with a leprosy case during the course of the study. In Nepal, a control group of 11 staff donors [nine Nepali/Indian, two Caucasian; eight male, three female; mean age 30 years (range 25–40)] who were leprosy free at the time of testing was recruited. All were currently working in the leprosy hospital so would be expected to have greater contact with *M. leprae* than the average Nepali resident. Seventy-four leprosy patients, who were either untreated and just starting, or had recently started, MDT treatment, were recruited through the outpatient clinic of Anandaban Leprosy Hospital, Kathmandu, Nepal, over a 2-month period. A small blood sample was obtained from each study subject following their informed consent. All subjects were subsequently tested once a month (when possible) over a 6-month period, in both UK and Nepal. Patients were retested on subsequent visits to the hospital, when detailed clinical records were taken and signs of onset of type 1 or 2 reactions were noted. Plasma isolated from the patient blood samples was tested for anti-PGL-1 IgM antibodies using the standard technique for detection in serum<sup>20</sup> (paired serum and plasma samples were tested and found to be comparable). Ethical permission for the study was obtained from the Ethics Committees at the LSH&TM, and Anandaban Hospital.

### WHOLE BLOOD ASSAYS

Whole blood assays, lymphocyte proliferation measurements and IFN $\gamma$  ELISAs were carried out as previously described.<sup>8</sup> Whole blood was diluted 1 in 10 with RPMI tissue culture

medium (Gibco BRL, Paisley, Scotland) supplemented with 100 IU penicillin/100 µg streptomycin/2 mM L-glutamine/ml (all Gibco), and incubated with antigen, and positive and negative control stimuli, in triplicate tissue culture wells. The cultures were pulsed with tritiated thymidine ( $^3\text{H}$ )TdR after 6 days incubation, harvested and counted in a scintillation counter. In the UK study, triplicate culture supernatants were harvested on day 6, pooled and frozen at  $-20^\circ\text{C}$  for batch testing for IFN $\gamma$  by ELISA at the end of the study. Total and differential leukocyte counts were performed on each blood sample and the number of lymphocytes per culture for each subject was calculated.

#### ANTIGENS

Cultures were incubated with *M. leprae* sonicate (MLS, batch CD215; Dr R. Rees, NIMR, Mill Hill, UK) and *M. tuberculosis* PPD (Statens Serum Institut, Copenhagen, Denmark). The mitogen phytohaemagglutinin (PHA; Sigma, Poole, UK) and tissue culture medium were used as positive and negative controls, respectively. In the UK study, recombinant *M. leprae* 30/31 kD protein (Ag 85B; kindly provided by Dr B. Wieles, Leiden, The Netherlands) and *M. tuberculosis* 70 kD protein (*Mtb* 70 kD; MT-70-6A, Dr J. van Embden, Bilthoven, The Netherlands) were also tested. The same batches of antigens were used throughout each study. The antigens were initially tested at 1, 10 and 30 µg/ml, and PHA at 5 µg/ml.

#### DATA ANALYSIS

Proliferation results are presented as the mean ( $^3\text{H}$ )TdR incorporation of triplicate cultures, after subtraction of background counts in the negative control wells (dDPM). The threshold for a positive response (calculated as  $2 \times \text{mean SD}$  above the mean negative control culture responses in 37 subjects; data not shown) was 500 dpm. IFN $\gamma$  responses are presented as mean IFN $\gamma$  measurements (IU/ml) of duplicate ELISA wells, after subtraction of any background measurement in the negative control culture supernatant (undetectable in the majority of cultures); a measurement greater than zero was regarded as a positive IFN $\gamma$  response. The variation in dpm measurements in triplicate cultures was calculated for one subject (UK/donor 1, in response to PPD) and found to be 5% on average. Anti-PGL-1 antibody measurements are presented as mean optical density (OD) at 492 nm. Interplate variation was controlled by the inclusion of positive and negative control samples on every plate, and was found to be less than 10%.<sup>20</sup>

Variation in responses over time, to each antigen in each subject tested at least three times, was calculated as a coefficient of variation (CV), where

$$\text{CV} = \frac{\text{SD (sequential response measurements)}}{\text{mean (sequential response measurements)}} \times 100\%$$

Other statistical tests are detailed where used.

## Results

#### VARIATION OVER TIME OF DOSE RESPONSE TO ANTIGEN IN UK SUBJECTS

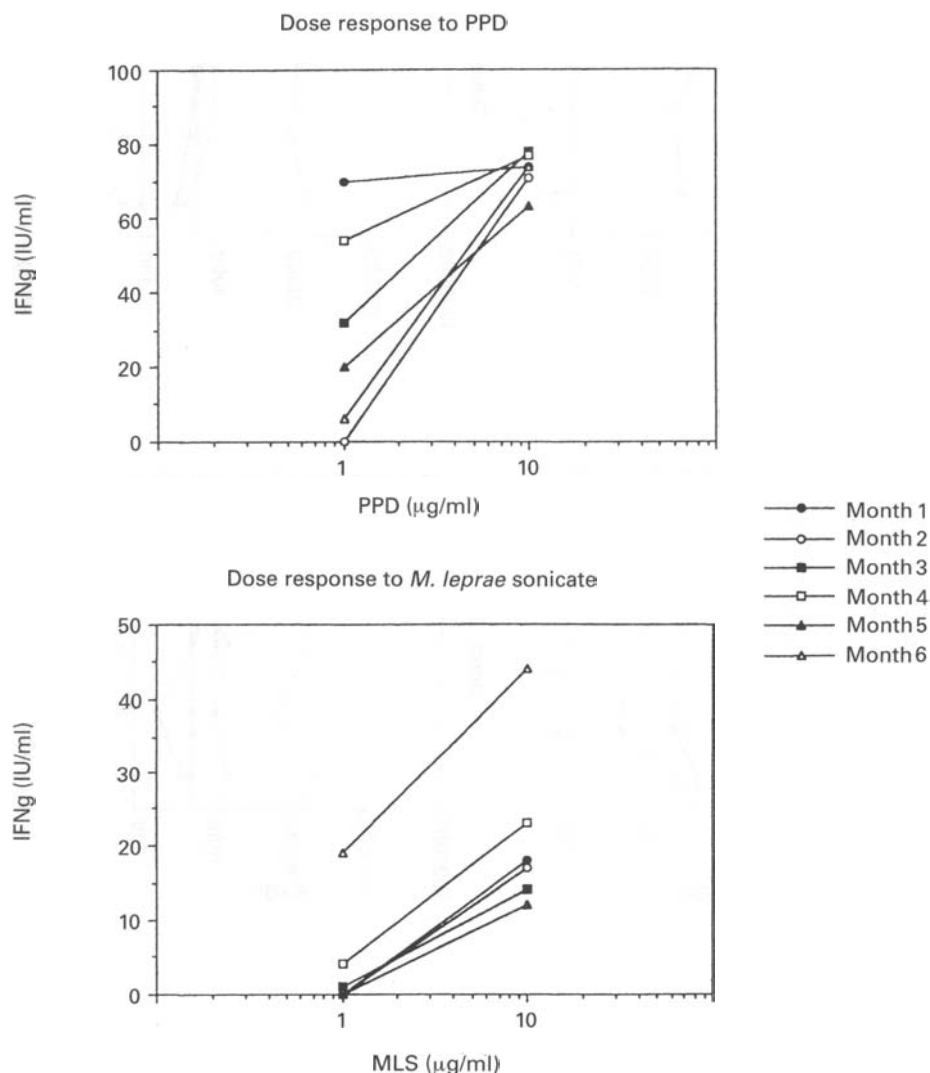
The optimal dose was found to be 10 µg/ml for all antigens (results not shown), although most subjects made a response to antigen at 1 µg/ml. Subjects in the UK study were routinely tested



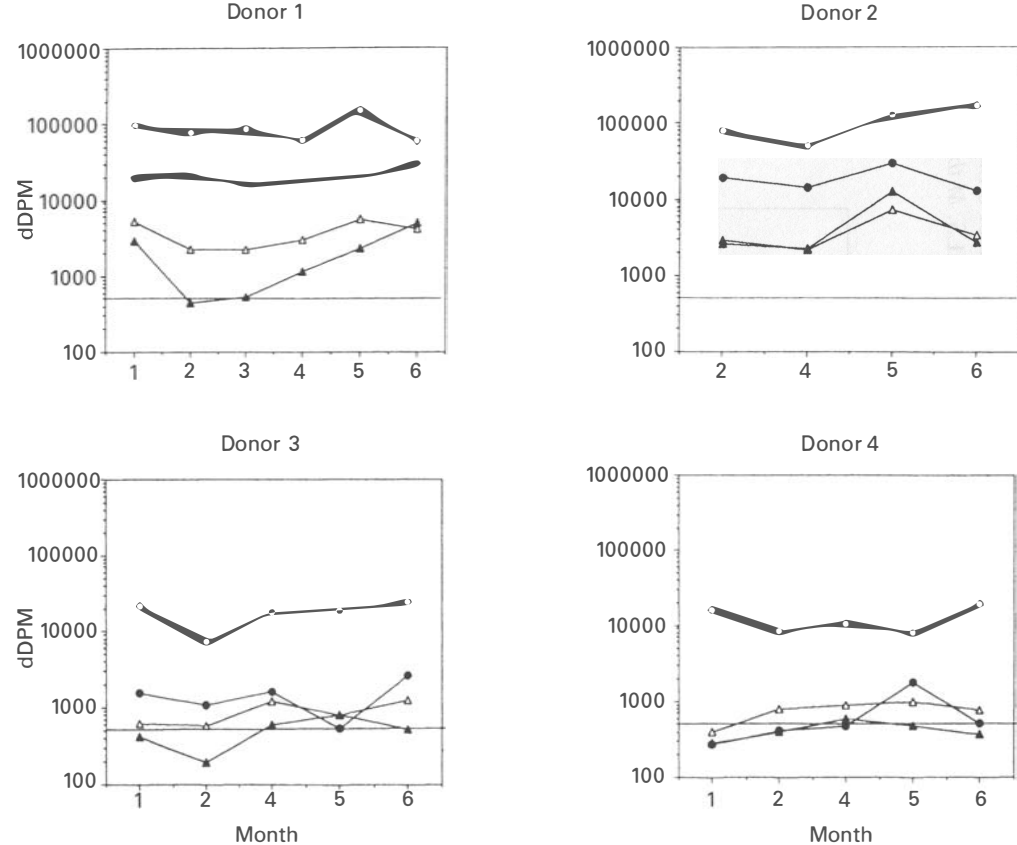
with antigen at 1 and 10  $\mu\text{g/ml}$  to assess whether the dose-response profile changed over time. Figure 1 shows results for  $\text{IFN}\gamma$  responses to PPD and MLS in one subject, tested once a month for 6 months. The dose response to PPD did appear to vary, with the response to 1  $\mu\text{g/ml}$  relatively higher on some months; however, for responses to both PPD and MLS, the optimum dose was always 10  $\mu\text{g/ml}$ .

#### VARIATION IN RESPONSES TO ANTIGEN AND MITOGEN IN CONTROL SUBJECTS

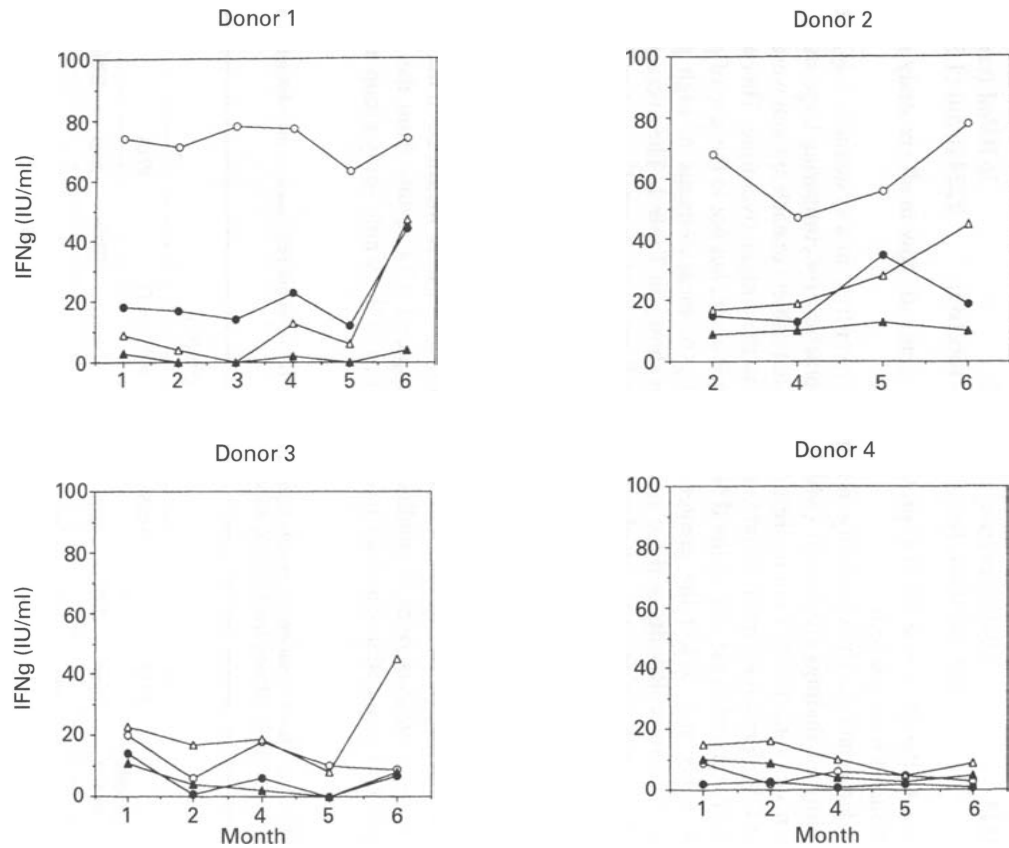
The six healthy UK control subjects were tested over the course of 6 months. Proliferation responses (Figure 2) and  $\text{IFN}\gamma$  responses (Figure 3) to PPD, MLS, *M.tb* 70 kD protein and



**Figure 1.** Dose response to PPD and *M. leprae* sonicate (MLS) at 1 and 10  $\mu\text{g/ml}$ , in whole blood cultures from one UK donor tested at monthly intervals, measured by  $\text{IFN}\gamma$  production (U/ml) in day 6 culture supernatants.



**Figure 2.** Proliferation responses (dDPM) in whole blood cultures from four UK donors, tested repeatedly over the course of 6 months. Cultures were stimulated with *M. leprae* sonicate (●), *M. leprae* 30/31 kD protein (▲), PPD (○) and *M. tuberculosis* 70 kD protein (△) at 10 µg/ml, and proliferation measured after 7 days culture. The positive response threshold (500 dpm) is indicated by a line.



**Figure 3.** IFN $\gamma$  responses (IU/ml) in whole blood cultures from four UK donors, tested repeatedly over the course of 6 months. Cultures were stimulated with *M. leprae* sonicate (●), *M. leprae* 30/31 kD protein (▲), PPD (○) and *M. tuberculosis* 70 kD protein (△) at 10  $\mu$ g/ml, and IFN $\gamma$  measured by ELISA in 6 day culture supernatants.

*M. leprae* 30–31 kD protein are shown for four subjects tested four or more times during the study. For both IFN $\gamma$  and proliferation measurements, donors 1 and 2 made consistently high or moderate responses to PPD, MLS and *Mtb* 70 kD, while donors 3 and 4 were consistently moderate responders to PPD and low or non-responders to MLS and *Mtb* 70 kD. The group average proliferation (dpm) and IFN $\gamma$  (IU/ml) responses to each antigen were:

PPD	67,421 dpm (range 12,409–112,411);	46 IU/ml (range 5–91)
MLS	11,079 dpm (range 0–20,269);	20 IU/ml (range 2–60)
<i>Mtb</i> 70 kD	2,048 dpm (range 0–3,945);	26 IU/ml (range 11–52)
PHA	124,765 dpm (range 87,603–166,169);	2224 IU/ml (1,320–2,901).

Responses to the *M. leprae* 30/31 D protein were generally low in all six study subjects and were excluded from analysis.

In order to analyse the variability in response over time in a systematic way, rather than representing the findings graphically (which is impractical for comparing large study groups), coefficients of variation (CV) of the response in each subject to each antigen were calculated. A higher CV represents a more variable response to the antigen over time. This was found to be inversely related to the size of the IFN $\gamma$  response made, but not to the size of proliferation response—this may reflect the greater variability of measurements of high proliferation responses in culture. Results of these analyses are shown in Table 1. The average CV for all six donors to each antigen was calculated, as an indication of ‘normal’ variation in responses to each of these mycobacterial antigens in UK donors.

Proliferation responses were less variable than IFN $\gamma$  responses (paired *t*-test, *p* = 0.015), and may therefore be a more reproducible measure of the T-cell response to *M. leprae* antigens in longitudinal studies. We have previously found that proliferation and IFN $\gamma$  responses parallel each other in these donors (data not shown).

Proliferation responses to PPD and MLS, and to PHA, were measured in the group of 11 Nepali control subjects over 6 months, and compared with results from the UK cohort. Average CV of sequential responses to each antigen in the whole study group are shown in

**Table 1.** Coefficients of variation for proliferation and interferon-gamma responses to mycobacterial antigens in sequential whole blood cultures from six UK donors

Donor	Number of tests	CV (%)								Cell no.
		PPD		MLS		Mtb 70 kD		PHA		
		dpm	IFN $\gamma$	dpm	IFN $\gamma$	dpm	IFN $\gamma$	dpm	IFN $\gamma$	
1	6	37	7	23	55	61	130	24	42	27
2	4	47	22	41	49	58	47	13	27	33
3	5	35	48	52	100	36	61	17	17	30
4	5	39	55	—	46	—	41	30	36	5
5	3	13	14	11	31	21	45	16	53	53
6	3	26	11	86	88	61	52	7	69	23
Group average		33	26	42	61	47	63	18	41	28

dpm = % coefficient of variation of proliferation measurements over sequential tests.  
IFN $\gamma$  = % coefficient of variation of IFN $\gamma$  measurements over sequential tests.  
cell no. = % coefficient of variation of mean lymphocyte number per culture.  
— = no detectable response so CV could not be calculated.

**Table 2.** Variation of sequential measurements of proliferation responses to mycobacterial antigens in Nepali healthy controls and leprosy patients

Group	<i>n</i>	PPD x	Mean % CV	MLS x	Mean % CV	PHA x	Mean % CV
Endemic controls	11	73,600	37	15,166	69	78,017	29
Leprosy patients:							
TT/BT (no reaction)	7	58,629	49	20,674	57	67,755	40
BB/BL/LL (no reaction)	5	30,128	62	0	—	69,561	15
Total patient mean			55		—	—	27

x = mean proliferation measurement (dpm) of sequential test cultures.

% CV = % coefficient of variation of proliferation measurements over sequential tests.

Table 2. The average variation observed in this group of hospital staff control donors was similar to that seen in the UK donors to PPD, but higher to MLS (and PHA).

#### VARIATION OF LYMPHOCYTE NUMBERS IN CULTURE

The lymphocyte number in whole blood cultures is not routinely estimated. To investigate whether this may influence magnitude of responses, lymphocyte numbers in culture were counted at each test point, and are summarized in Table 1 for the UK control subjects. The average lymphocyte number per culture was  $3.64 \times 10^4$ ; the average CV of lymphocyte number for the six subjects over the testing period was 28%. Lymphocyte number was compared with proliferation and IFN $\gamma$  responses to the antigens. There was no positive correlation between lymphocyte number and responses to any of the antigens, in either the UK or the Nepali control groups. Responses to PHA also did not correlate with lymphocyte number; this may be due to responses being measured on day 6, after the optimal day of response to PHA (day 3).

#### RESPONSE VARIATION IN NEPALI LEPROSY PATIENTS

Of the initial cohort of 74 leprosy patients recruited into the study, 22 returned and were tested at least three times. Nine patients were classed as 'paucibacillary' (PB: four TT, five BT), and 13 as 'multibacillary' (MB: three BB, seven BL, three LL). These subjects were all Nepali, 19 male and three female, mean age 31 years (range 12–68 years), who had received 0–8 doses (median one dose) of MDT prior to the study; 10 patients were untreated. Patients who experienced reactions were not retested until at least a month after finishing prednisolone (or thalidomide) treatment, to allow the immunosuppressive effects of the drug to diminish. Two patients (one BL, one LL) developed or remained in type 2 reaction during the study and were excluded from analysis.

Twelve patients (seven PB, five MB) remained free of the clinical signs of reaction during the study. Both PB and MB patients made similar responses to PHA, but the MB group made overall lower responses to PPD, and low or no responses to MLS (Table 2). Compared to the Nepali endemic group, response variation in these patient groups was higher to PPD, slightly lower to MLS (PB patients) and similar to PHA. Therefore, active clinical leprosy did not appear to increase the variability of T-cell responses to *M. leprae* above what would be

expected in healthy subjects. Increases in T-cell response prior to onset of reaction would have to be significantly above these 'normal' limits of variation to be noticeable. Development of a positive MLS response may be significant in MB patients who made low or no responses to this antigen.

Four patients were in type 1 reaction on their first test; their responses to PPD and MLS over the course of the study are shown in Figure 4(a). Three patients showed higher responses to MLS at this time than on subsequent testing which was significantly greater than would be anticipated from normal variation' (BT28–74% higher, BB2–300% higher, BL14—a positive response which then became negative). One patient (BL29) made no response to MLS both during and after reaction. Responses to PPD were either similar or lower during/post-reaction; PHA responses were within the limits of anticipated variation throughout. Change due to reaction is, however, more difficult to assess in these patients in whom pre-reaction data is lacking.

Four patients developed type 1 reaction during the study (Figure 4b). One patient (BT27) showed an increase in proliferation response to MLS of over 200% compared to responses preceding and following the reaction. Responses to PPD and PHA did not vary significantly in this subject. The MB patients in this group showed consistently low or negative responses to MLS. Responses to PPD and PHA were relatively stable in these subjects, apart from patient BB4, who showed a distinct increase in response to PPD at the time of the reaction.

Although based on a small group of patients, these results suggest that detectable increases in *in vitro* responses to *M. leprae* may be observed in PB leprosy patients undergoing type 1 reaction, but multibacillary patients continue to make negative responses.

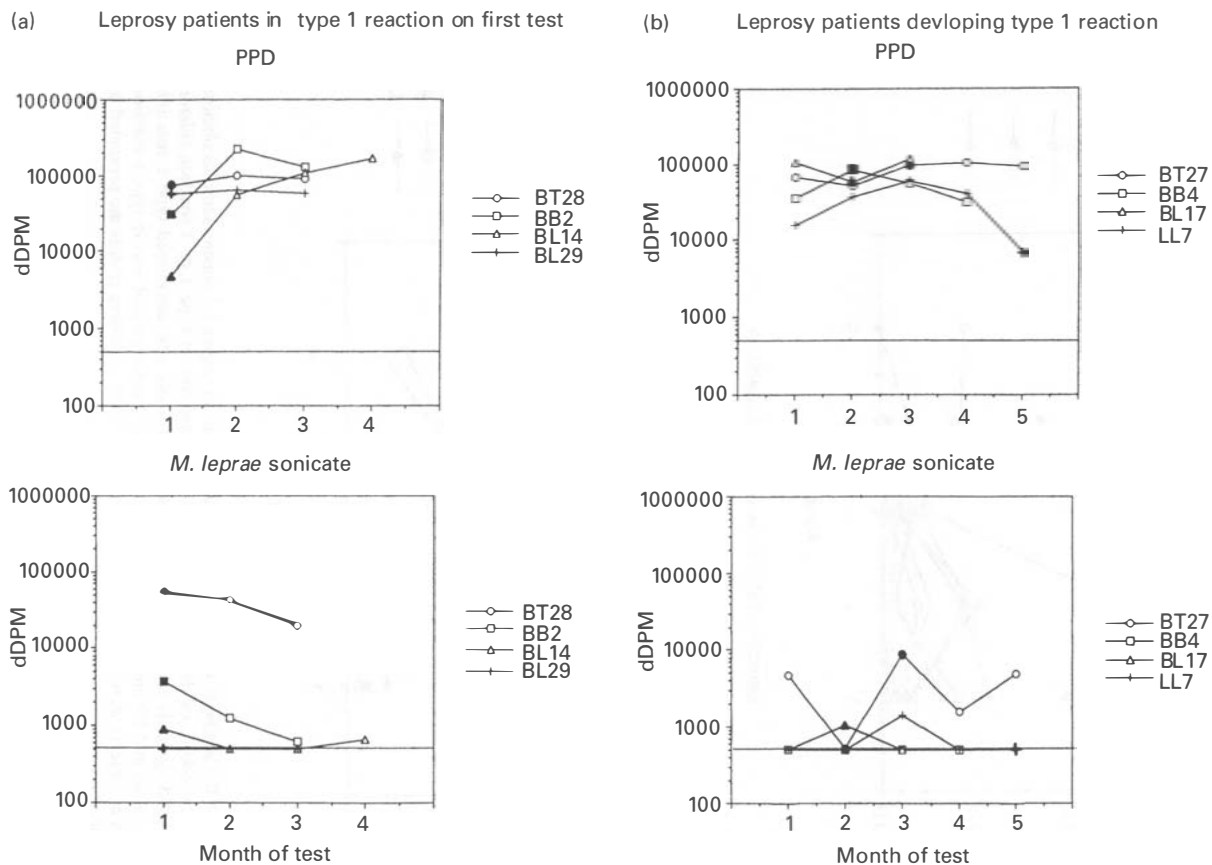
#### SEQUENTIAL ANTI-PGL-1 ANTIBODY RESULTS IN NEPALI LEPROSY PATIENTS

Figure 5 presents the anti-PGL-1 antibody titres measured in the same patients over the course of the study. In the group of patients who did not go into reaction (Figure 5a), anti-PGL-1 titres were generally higher in the MB patients, lower in the PB patients, and showed an overall fall in titre with treatment in agreement with previous studies<sup>24</sup>, although this was not a significant decline. All patients had only recently started treatment, apart from one LL patient, who had received eight doses of MDT. Of the patients who developed type 1 reaction during the study (Figure 5b), anti-PGL-1 antibody responses were not obviously higher prior to or during the reaction. Two patients had received MDT treatment prior to the study: BL17 (six doses), who showed signs of reaction on test 2, and LL7 (eight doses), who showed signs of reaction on his final test. Measurement of anti-PGL-1 antibodies cannot therefore be used to predict type 1 reactions.

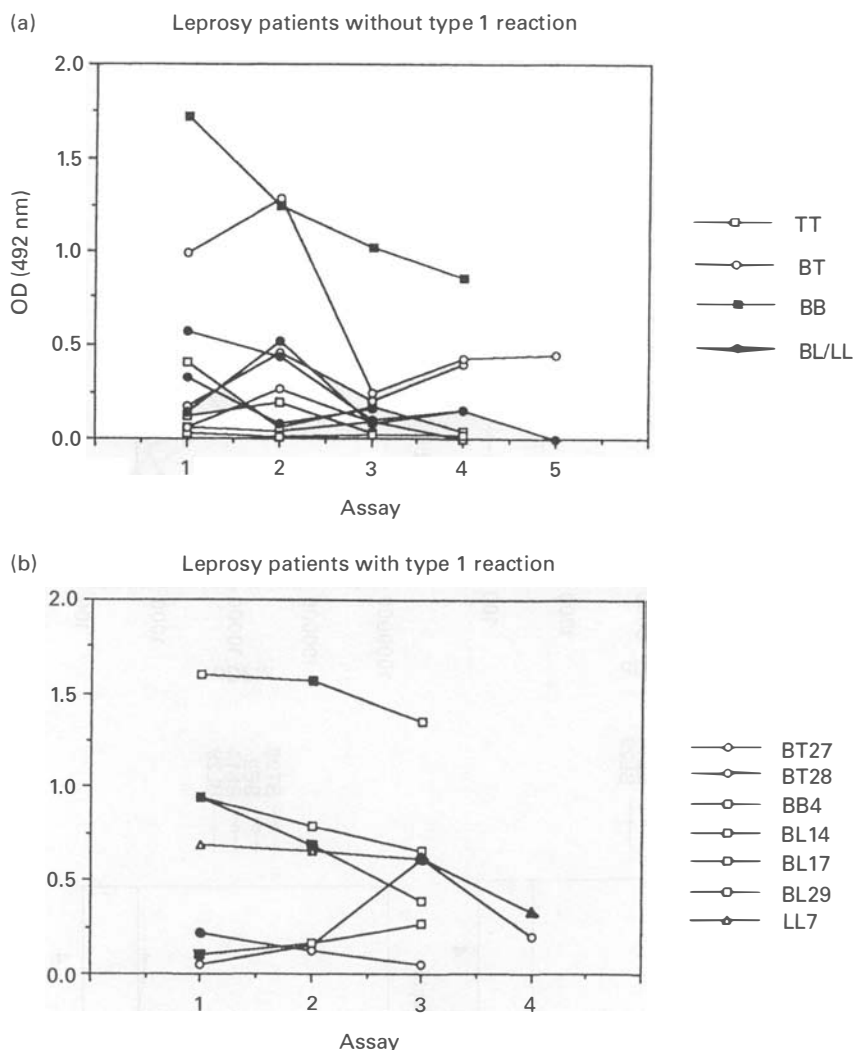
## Discussion

The use of a whole blood assay, requiring only a small volume of blood, made possible longitudinal studies of *in vitro* T-cell (proliferation and IFN $\gamma$ ) responses to mycobacterial antigens and mitogen in the UK and Nepal. The assay itself was found to be reproducible, with a standard dose of antigen (10  $\mu$ g/ml) giving consistently optimal responses. Variations in responses were found to be independent of lymphocyte number in culture, in agreement with other studies.<sup>25,26</sup>

Previous studies have used separated peripheral blood mononuclear cell (PBMC) assays



**Figure 4.** Proliferation responses (dDPM) to PPD and MLS (at 10  $\mu$ g/ml) in sequential whole blood cultures from leprosy patients (a) in type 1 reaction on the first test or (b) who developed type 1 reaction during the course of the follow-up study. Each symbol represents one patient (identified by leprosy classification and ID number); solid symbols indicate that the patient was in type 1 reaction at the time of testing.



**Figure 5.** Anti-PGL1 antibody measurements (OD at 492 nm) in sequential plasma samples obtained from leprosy patients during the course of the study. (a) Patients who were free of type 1 or 2 reaction (identified by leprosy classification, solid symbols represent MB patients); (b) patients who developed type 1 reaction (identified by leprosy classification and ID number, solid symbols indicate that the patient was in type 1 reaction at the time of testing). As a few subjects were not tested for anti-PGL1 on every occasion, results are presented by 'assay' rather than by 'month of test'.

to carry out longitudinal studies of T-cell proliferation responses to *M. leprae* in leprosy contacts at risk of developing leprosy,<sup>27</sup> leprosy patients receiving immunotherapy<sup>28</sup> and leprosy patients undergoing reactions<sup>23</sup> to investigate changes in the immune response that correlate with alterations in the clinical symptoms of the subject. However, none to our knowledge have studied the variation in the 'normal' T-cell response to *M. leprae* antigens in healthy subjects, which must be taken into consideration both when interpreting results from



single measurements collected in cross-sectional studies of T-cell immunity in leprosy, or when interpreting changes in immune responses during clinical leprosy, for example in reaction studies. In addition, results are usually presented graphically, and analysed as changes between positive and negative responses to *M. leprae*. In this study, we have attempted to quantify variability in the magnitude of T-cell responses over time, so that differences between study groups could be analysed for significance.

The study of control donors in the UK found responses to *M. leprae* sonicate, *M. tuberculosis* PPD, and the mitogen PHA, to be relatively stable in that an individual consistently responded or did not respond. Calculation of coefficients of variation for this data showed variation of the magnitude of response to be approximately 30–40%. IFN $\gamma$  responses were found to be more variable than proliferation responses.

Control subjects in a leprosy endemic area of Nepal showed more variable proliferation responses to MLS and mitogen than the UK controls. As identical protocols were used in each location, greater variation in Nepal may reflect current exposure to *M. leprae*, or other infectious agents. This baseline variation was comparable with that seen in responses of leprosy patients who were free of reactions during the study. Active leprosy cases receiving chemotherapy might be expected to show more variable responses due to the release of antigens from dead *M. leprae* in the tissues (one mechanism proposed to underlie reaction onset), but this did not appear to be the case.

A significant increase in response to *M. leprae* sonicate above the baseline level of variation was seen in two paucibacillary leprosy patients who experienced type 1 reactions during the study. In one case, this coincided with a significant increase in response to PPD, otherwise PPD responses were consistently positive but did not increase significantly in these patients. No significant changes were observed in mitogen responses in any of the patient groups. This indicates that the response to *M. leprae* in particular rather than mycobacterial antigens or mitogen in general may be predictive of reaction onset.

The multibacillary patients did not respond to *M. leprae* sonicate even when in reaction, which indicates that *in vitro* responses to *M. leprae* may not be useful for anticipating onset of reactions in multibacillary patients. It may be that significant inflammatory changes at reaction onset are only apparent in the T-cells of the lesions, rather than in the blood, of these patients. Anti-PGL-1 antibody titres did not change in the patients undergoing reaction, so although high titres are associated with a tendency to develop reactions,<sup>20,21</sup> increased anti-PGL-1 antibody titres would not appear to be directly predictive of reaction onset.

If the increase in response to *M. leprae* observed in the two BT leprosy patients in reaction in this study is a real effect, it remains to be established if this occurs before the clinical appearance of a reaction; neither of these patients showed increased responses a month prior to reaction becoming apparent. If the two events occur simultaneously, immediate treatment with steroid would still not completely prevent nerve damage due to reaction. In addition, close monitoring of patients using a test which does not give a result for a number of days is not feasible in an area such as Nepal. Patients often make long journeys for brief visits to the clinic and may not return for several months, making sequential testing of patients difficult as this study found. In conclusion, a whole blood assay to measure *in vitro* T-cell responses to *M. leprae* antigens is a useful and simple way of monitoring cell mediated immune responses in leprosy patients, but is probably not practical as a diagnostic tool for predicting and preventing reactions in leprosy.

This study has clarified the 'normal' baseline variation in T-cell proliferation and IFN $\gamma$  responses to *M. leprae* antigens in a range of subject groups. The whole blood assay could

now be used in a larger longitudinal study of leprosy patients, possibly using more defined antigens and a range of cytokines as response markers, and in conjunction with studies of immunological markers in the lesions, to investigate in more detail the immune mechanisms in leprosy that may help to understand the onset, and eventually reduce the incidence, of type 1 and 2 reactions.

## Acknowledgements

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*SPECIAL ARTICLE*

## Journals on the Net: some sites reviewed

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### Introduction

Following the recent article on 'Leprosy and the Internet' by J. S. Gilbody (*Lepr Rev*, 1997; **68**: 367–370), it was felt that readers of *Leprosy Review* might find it useful to have regular updates on what is available, not just in the area of leprosy, but in medicine generally. This is the first of these updates, and it reviews four websites operated by medical journals, the *British Medical Journal*, the *Lancet*, the *New England Journal of Medicine* and, last but not least, *Leprosy Review's* own website. The review concludes with some thoughts on the advantages and disadvantages of electronic journals for the developing world.

### British Medical Journal (<http://www.bmj.com/index.shtml>)

The *British Medical Journal* has recently provided free access to all published articles on its website. In a recent Editorial, Tony Delamothe, *BMJ's* Web Editor, and journal Editor Paul Smith said: 'Three years ago, it was hard to find a medical journal on the internet. Now most have websites, providing selections from their paper journals in electronic form. ....the *BMJ* joins the *Lancet* and a host of specialist journals in taking the obvious next step: providing the full text of the paper journal online. Soon most other medical journals interested in their long term survival will follow suit.'

The *BMJ's* website is attractively laid out, and easily accessible. No registration is required. Readers can browse through the current issue of the journal, or search for topics by keyword, date or volume and page number. If a search of the *BMJ's* archives is unsuccessful, there is the opportunity to look in the websites of other journals. Readers can opt to have the Table of Contents e-mailed to them free each week, and may also request information only on topics in which they are interested – an attractive option for specialists in relatively rare topics such as leprosy!

The *BMJ's* policy is to make the electronic journal their main route for publication, although they will continue to publish a paper version in tandem. They cite the advantages of virtually unlimited space on the Web, so that they can promise earlier publication of particularly interesting and topical articles. Use of the Web will also result in considerable savings in postal and printing charges.

At present, access to the site is free; however, the Editors recognize that eventually, as readers change from the paper to the electronic version of the journal, they will either have to charge a fee for access, or seek sponsorship.

One encouraging sign is the fact that the BMJ Publishing Group are already thinking of how they might provide the developing world with access to their journals. A few lines of computer programming could allow free (or heavily discounted) access to computers from selected countries and, unlike discounts on paper subscriptions, the gesture would cost publishers nothing.

**Lancet** (<http://www.thelancet.com/>)

The *Lancet's* website is largely accessible to non-subscribers to the paper journal, but registration is required in order to use it, although there is no fee for registration. Users are presented with an on-screen registration form, asking for name, contact details, job and specialist interests. When this has been filled in, the user can choose an on-screen name and password, which is needed to access the site in future.

The site has several areas. The Journal area enables the user to browse the current issue, supplements, back issues and press releases. The Discussion Groups provide a forum for subscribers to debate material published in *The Lancet*. Topics currently covered include tamoxifen and breast cancer, malaria and the UK National Health Service, but new subjects are constantly being introduced. The Arcade provides advertising, job opportunities, subscription details, links to other websites and media information. Information for Authors has instructions for submitting papers and contact numbers for *Lancet* staff. There is also a Search area, for locating articles on particular topics.

Subscribers to the paper journal have access to a special area in the Journal area, which contains the complete text of each article, but the 'free' area is quite comprehensive, and includes all the other facilities. It should be sufficient for most casual users.

The *Lancet* site is aimed more at Western European readers, and has not so far identified the developing world as a potential market.

**New England Journal of Medicine** (<http://www.nejm.org/content/index.asp>)

This journal's website also has two parts: a free, unrestricted area for all users, and a subscriber-only area. The subscriber-only area offers subscribers access to the complete text of the journal, including review articles, for all issues since 1993. Additional features for subscribers are a full-text search capability and a personal archive for saving links to articles and searches of interest. Using free software, subscribers can download articles and print them out in a format that is virtually identical to that of the printed pages.

For non-subscribers and casual users, the unrestricted area on the web site offers an electronic version of the Journal that includes all abstracts, editorials, letters to the editor, and book reviews, as well as classified advertisements and customer services. As with the *Lancet* and *BMJ*, readers can opt to have the Table of Contents e-mailed to them free each week. They can also order offprints of individual articles for a fee of \$10 per article.

**Leprosy Review** (<http://www.lepra.org.uk/>)

This website is operated by the leprosy charity Lepra, and includes the Table of Contents,

Editor's Choice and Editorials from *Leprosy Review*. Users have access to back issues, but there is currently no search facility. The possibility of providing free electronic access to the full text of all original articles in *Leprosy Review* is currently under review.

### **Electronic journals and the developing world**

Subbiah Arunachalam, an Indian information scientist, believes that the Internet could widen the information gap between the developed and the developing world before it reduces it. Poor connections mean that even those with Internet access must spend hours downloading material that would take only minutes for those in the developed world who have the best access. Paradoxically, however, the Web could also prove more helpful for the information poor than any number of out of date medical journals and books sent to the developing world, where many libraries and institutions lack the resources to subscribe to current copies. Medical libraries in these countries would be far more likely to acquire computers with Internet access than to fill their shelves with vast quantities of information, much of which may not be relevant to their researchers.

Of the sites reported here, only the *BMJ* seems to have considered providing free, or reduced subscription, access to countries in the developing world. Hopefully, as electronic publishing becomes even more popular and more flexible, more journals will take up this option.

## *FURTHER EDUCATION*

# Indications for surgery in leprosy

MARCOS VIRMOND

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## Introduction

### *What does surgery offer?*

1. Correction of paralytic deformity by reconstructive surgery.
2. Correction of primary deformity by plastic surgery.
3. Prevention of worsening of deformity.
4. Prevention of deformity by nerve decompression.
5. Early healing of plantar ulcers.

### *Common sites of nerve damage and deformity*

1. Ulnar nerve at elbow – clawed fingers and thumb.
2. Median nerve at wrist – fallen back or ‘ape’ thumb.
3. Lateral popliteal nerve at fibular neck – drop-foot.
4. Posterior tibial nerve in tarsal tunnel – clawed toes.
5. Facial nerve at zygomatic arch – lagophthalmos.

### *Common reasons for referring to a surgeon*

1. Paralytic motor deformities of hand, foot and eyelids.
2. ‘Neuritis’ of a trunk nerve not improving after 2 weeks of medical treatment.
3. Primary deformities of nose, face and eyebrows.
4. Non-healing plantar ulcers.

### *Common symptoms*

1. Trunk nerve enlargement and tenderness.
2. Pain in the nerve, at rest and on stretch.
3. Reduction or loss of sense of touch, pain and temperature.
4. Loss of sweating.
5. Motor weakness and increasing paralysis.
6. Nerve abscess.

## Rationale for surgery

Leprosy affects nerves, skin and other soft tissues. Delay in treatment allows development of deformities indirectly by nerve damage (paralytic deformities and sensory loss) and directly

by tissue damage (primary deformities). All these deformities can be corrected by surgery while sometimes nerve damage can be reversed or further damage prevented by nerve surgery. Surgery at any stage can stop further worsening of a deformity. Surgery gives best results when performed early, before secondary changes develop because of the abnormal structure. However, surgery is available only at special centres, so a proper referral process is essential.

### **How are deformities caused?**

Nerve trunk damage produces deformities indirectly through sensory loss and loss of motor power. Loss of pain and other sensation increases the vulnerability to injury and infections, and results in ulcers in the palm and sole. Loss of motor power causes muscular imbalance leading to deformity. For example, loss of flexor power at the metacarpophalangeal (MP) finger joint causes hyperextension of the MP joints and flexion of distal joints—the clawing deformity. The disability here is of grasp, because the fingers now roll over instead of flexing first at the MP joints. Secondary to these deformities, because of altered position and dynamics, there occur other complications like joint contractures, adaptive shortening of flexors, ulceration at knuckles and under the metatarsal heads and finger tip flexion.

The primary deformities, like depressed nose and loss of eyebrows, are seen mainly when treatment is delayed in lepromatous patients. These can be well corrected by plastic surgery.

### **Common reasons for referral to a surgeon**

There are a group of conditions in leprosy patients that cannot be adequately managed by basic health personnel and should be referred to a surgeon. The main conditions are listed below.

#### **PARALYTIC DEFORMITIES AND DISABILITIES OF HANDS**

Definite clawing of the fingers and lack of opposition of the thumb that cannot be restored by physiotherapy. This condition requires tendon transfer procedures. Surgery can improve clawing of the fingers, allowing patients to grasp large objects and improving pinch with the non-paralysed thumb, thus restoring adequate hand function to daily activities and labour.

#### **PARALYTIC DEFORMITIES AND DISABILITIES OF FEET**

Definite drop foot and clawed toes are conditions which health education and prevention of disabilities can prevent from worsening, but only surgery can restore dorsiflexion of the foot and reverse clawing of the toes. Tendon transfers are the procedure of choice to restore dorsiflexion to a dropped foot, with the aim of restoring the normal gait, thus preventing destructive ulcers in the lateral border and fixed deformity of the ankle with equinus–varus. Tendon transfers can also restore, to some extent, a straight position of the toes, preventing ulcers in the dorsum. Correction of the clawed toes leads also to an improved distribution of pressure under the metatarsal heads, so preventing plantar ulcers in anaesthetic feet.



## LAGOPHTHALMOS

Failure to close the eyes is a serious and threatening condition. Exposure keratitis due to drying of the cornea can lead to corneal ulceration and, ultimately, to blindness. This condition can be treated, for a while, by oil drops and corrective lenses. Surgery can offer a more definite protection of the exposed eye. Temporal muscle transfer, tarsal sling and tarsorrhaphy are procedures that can provide an adequate and long standing protection to the cornea of an eye which cannot be closed due to paralysis of the orbicularis muscle.

## ULCERS AND INFECTIONS

Although plantar ulcers are commonly treated by dressings in ambulatory patients, there are some situations that call for surgical removal of dead bone and other tissues. A constantly discharging ulcer that does not heal despite adequate cleaning and dressings is a clear sign that some necrotic bone or infected tissue (tendon or capsule) is preventing ulcer healing and requires surgery. Deep spaces, synovial sheath and pulp infection in the hand are common situations that can go unnoticed due to loss of sensation and consequent absence to pain. These infections should be identified early and promptly treated by adequate surgical drainage to prevent its progression, which eventually leads to shortening of the fingers and severe skin contractures, i.e. a non-functional hand.

**Neuritis**

The trunk nerves in leprosy are involved at particular sites, where the nerve passes through a fibro-osseous tunnel. Here, the nerve is compressed when enlarged. The symptoms are due to inflammation and compression, pain, tenderness, stretch pain on flexion of the joint and sensorimotor and autonomic dysfunction distally. Early diagnosis and treatment can reverse the nerve damage. Sometimes, surgical decompression is required.

## COMMON SYMPTOMS

*Paralytic deformities of the hand*

Ulnar nerve paralysis:

1. Moderate abduction of the little finger.
2. Wasting of the hypothenar eminence and of the first web.
3. Inability to spread fingers.
4. Clawing of fingers, especially ring and little fingers.
5. Difficulty in grasping.
6. Sensory loss in little finger and half of ring finger.
7. Flexion of thumb tip during pinch.

Median nerve paralysis:

1. Progressive weakness of thumb opposition.
2. Wasting of thenar region.
3. Sensory loss in lateral two-thirds of hand.

Ulnar–median paralysis (commoner than median paralysis alone):

1. Clawing of all the fingers.

2. Loss of opposition.
3. Only key pinch is available, normal grasp and pinch are not available.
4. Sensory loss in the palmar aspect of the whole hand.

### *Paralytic deformities of the leg and foot*

Lateral popliteal nerve paralysis:

1. Wasting of anterolateral compartment of the leg.
2. Inability to dorsiflex the ankle and toes.

Posterior tibial nerve:

1. Wasting of the foot.
2. Inability to squeeze the toes together or to spread them.
3. Inability to keep the toes straight while elevating the heel.
4. Sensory loss in the sole (plantar aspect).

### *Facial nerve*

1. Inability to close the eyelids.
2. Dry eyes.
3. Sometimes corneal anaesthesia.

## **Prerequisites for surgery of primary deformities**

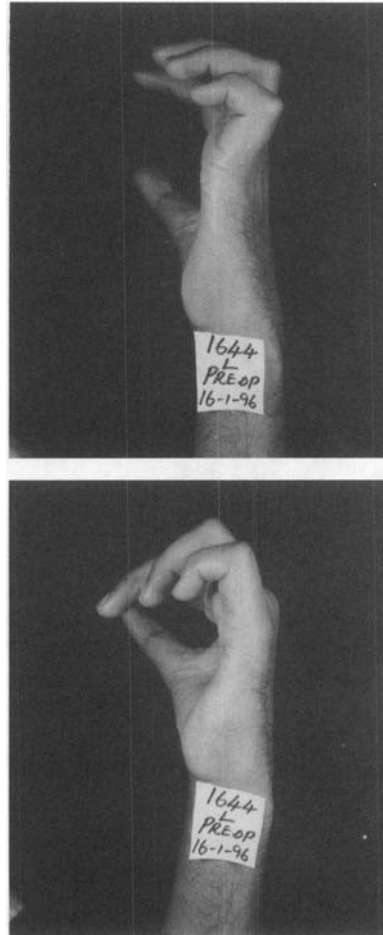
1. Adequate antileprosy treatment.
2. Bacterial index preferably 2 or less.
3. Adequate control of local infection.

The symptoms of paralytic deformities depend on the nerve involved and its distal distribution.

In the hand, ulnar nerve paralysis is the commonest, followed by the combined ulnar–median paralysis. In ulnar nerve paralysis (Figure 1), the medial one-third of the palm has sensory loss, while early mild abduction of the little finger is due to paralysis of the lumbrical and interossei and excessive action of the extensor tendon, which has a slight ulnarwards insertion of this finger (Wartberg’s sign). With progression of nerve damage, there is now increasing clawing of the fingers with MP hyperextension and flexion of the distal joints, because of unbalanced action of the extensors. All the interossei, which provide the normal flexor force at the MP joint, are paralysed here.

Isolated median nerve paralysis is uncommon. Usually, there is a combined ulnar–median paralysis (Figure 2), with clawing of all the fingers and loss of thumb opposition, which is a very disabling condition, as the patient cannot pick up objects. The whole palm of the hand also has sensory loss. This is a hand with hardly any function.

In lateral popliteal nerve paralysis, there is loss of function of the dorsiflexors. The result is inability to dorsiflex the foot and toes (drop foot). The gait is now with the forefoot striking the ground first—the high stepping gait. Often the evertors, the peronei muscles, are also paralysed, producing inversion deformity and later ulceration on the lateral border of the foot because of excessive pressure during walking and standing. Long-standing paralysis of these

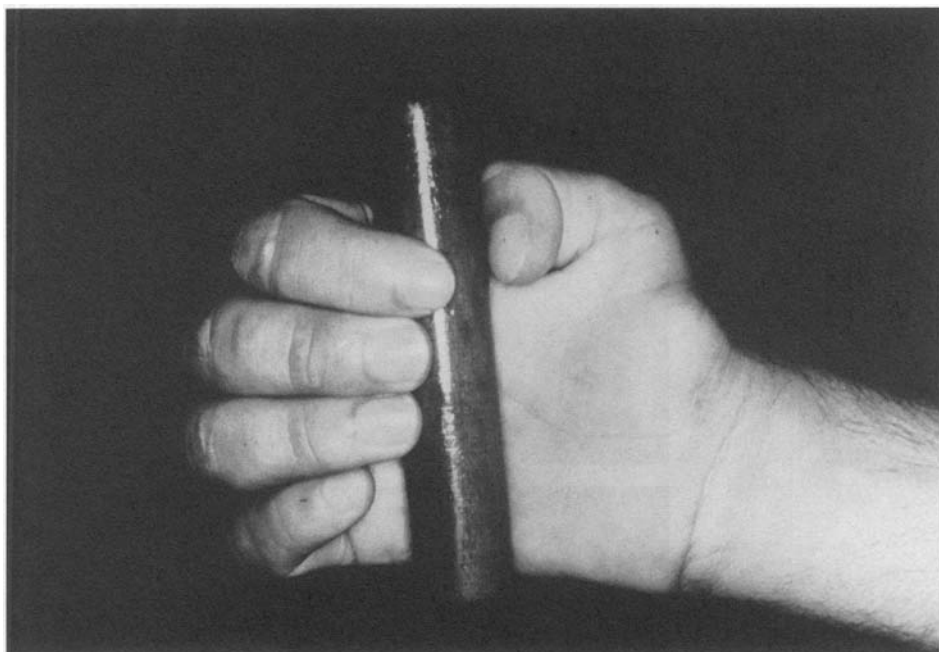


**Figure 1.** (a) Wasting of abductor digiti minimi, the lumbricals and interossei. (b) Clawing of the fingers with MP hyperextension and flexion of the distal joints.

muscles leads to rigid equinus varus deformity—the foot is fixed in plantar flexion with internal deviation.

In posterior tibial paralysis (Figure 3), the intrinsic muscles of the foot are paralysed, causing progressive hyperextension of metatarsophalangeal joints and flexion of the distal phalanxes (clawed toes). The condition of clawed toes has the same pathophysiology as clawed hand. The dorsum of the toes is exposed to excessive pressure as well, as the toe tip bends downward and is flattened because the toe pad is bearing at normal pressure. The toe tip is not anatomically designed to support pressure in the way that the toe pad is. With progression of the deformity, the metatarsal heads are overexposed in the plantar surface of the forefoot. Increased weight bearing in this area produces plantar ulcer. This is why clawed toes in an anaesthetic foot produce plantar ulcers in the forefoot.

In facial nerve paralysis, the eyelids are unable to close fully, the eye remains open, and becomes dry. Exposure keratitis may occur and lead ultimately to blindness. Thus this

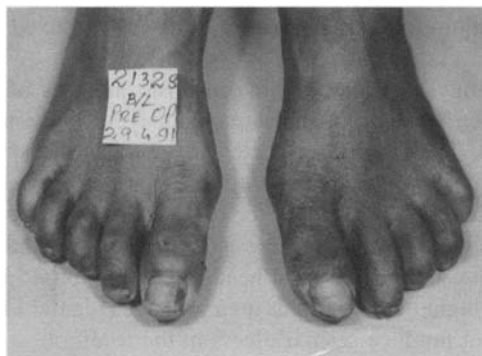


**Figure 2.** Loss of grip in combined ulnar/median paralysis (courtesy of F. Duerksen).

condition needs to be treated urgently to prevent damage to the eye. Early medical and physical therapy treatment is often successful, otherwise surgery is essential to save the eye.

### Evaluation and monitoring

This is done using graded nylon filaments for testing sensation. Motor power is tested by using the original or modified MRC scale. All clinical signs are also graded. Repeated monitoring is essential to observe progress, or otherwise, and take preventive action in time.



**Figure 3.** Posterior tibial nerve paralysis causing clawed toes (courtesy of D. Palande).

## Indications for surgery

### *Nerve surgery*

1. Inadequate improvement after 2 weeks of medical treatment.
2. Chronic nerve pain.
3. Nerve abscess.
4. Recurrent neuritis.
5. Ischaemia plantar ulcers or ulcers which are not healing.

### *Established paralytic deformities*

1. One year duration of paralysis.
2. Clawing of fingers, with or without clawing of the thumb.
3. Foot drop of 6 months or longer duration.
4. Clawed toes.
5. Lagophthalmos of longer than 1 month duration.

### *Ulcer surgery*

1. Non-healing ulcers.
2. Ulcers with septic or synovial discharge.
3. Ulcers with foot involvement.

### *Primary deformities*

Any primary deformity, once it has occurred, is irreversible and the patient should be referred for surgical evaluation to decide when surgery should be done.

Palatal and nasal deformities occur in neglected cases of lepromatous leprosy. Early prevention is by nasal care during treatment. Plastic surgery procedures can correct these deformities.

Facial wrinkles and sagging ear lobes result from subcutaneous infiltration and are easily corrected by face lift and plastic surgery.

Gynaecomastia occurs secondary to hormonal changes following testicular atrophy and treatment is surgical.

There is a group of uncommon conditions that can be corrected by surgery for functional and cosmetic reasons. Wrist drop alone is rarer than a triple nerve paralysis, which is a very disabling condition needing multiple tendon transfers for its correction. Club foot needs radical correction and fusion of joints. Sagging ear lobes, loss of eyebrows and gynaecomastia all need correction for psychological reasons. Nasal and palatal deformities are very disabling and corrective plastic surgery is essential. The results of surgery in all these conditions are excellent.

## Prerequisites for surgery

In all cases, before surgery is done, the paralysis should be of 1 year duration, i.e. irreversible, and the patient should be receiving multi-dose therapy and without recent reactions. The bacterial index of skin smears should be  $\leq 2$ . For primary deformities like those of the nose or palatal perforation, the disease must be inactive. In any surgery, there must not be any septic focus anywhere, as sepsis spoils the result completely.

Any established deformity is an indication, and the earlier the surgery is performed, the better is the result. For nerve surgery, the main indications are failure of medical and physical therapy, recurrent neuritis, intractable pain and a nerve abscess. For the posterior tibial nerve, decompression is not only of the nerve, but also of the blood vessels, and this improves the blood supply.

### Ulcer surgery

Surgery is required here mainly to remove a septic focus, open up any tracks, and to remove any dead bone. Sometimes, in cases of recurrent ulceration of the forefoot, metatarsectomy is needed, while for a heel ulcer, some flap procedures may be required. The mainstay for prevention and treatment of ulcer recurrence is care of the foot and proper orthotic devices, including footwear.

### Conclusion

Surgical treatment is essential to comprehensive care of the leprosy patient. Indications are clearly defined and should be known to all health personnel involved in leprosy. If surgical facilities are not available locally, an efficient referral system needs to be established.

### Further reading

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- Fritschi EP. *Surgical reconstruction and rehabilitation in leprosy*. Director for Southern Asia, Leprosy Mission, New Delhi, 1992.
- McDowell F, Enna C. *Surgical rehabilitation in leprosy*. Williams & Wilkins, Baltimore, 1974.
- Srinivasan H, Palande DD. *Essential surgery in leprosy – techniques for district hospitals*. World Health Organization, 1997.

## Letter to the Editor

### **EDITORIALS, SEPTEMBER 1997 BY W. C. S. SMITH, P. FINE & D. K. WARNDORFF**

Editor,

Both the Editorials by Drs Smith and Fine raise issues on 'incidence' in leprosy elimination programmes. One of the objectives of leprosy elimination campaigns is to reduce disease burden in the community by detecting backlog cases and treating all active leprosy cases, thus reducing the pool of infection in the community well before the target date. For the leprosy elimination campaign, elimination indicators (direct and indirect) described in the LEM guidelines of WHO should be adequate for assessment of all situations. As the measurement of incidence (with its severe limitations) is operationally not feasible at the moment, we have no alternative but to rely on prevalence and detection indicators. After elimination, we need to work towards leprosy eradication (zero leprosy), for which simple operational and epidemiological technology is still to be developed. At that time, emphasis on incidence will be more relevant. Similarly, zero disability will also have its importance.

I appreciate the concern expressed by Dr Fine and Dr Warndorff regarding the future of fundraising and the support for leprosy activities. There is a justification for reducing the infrastructure for leprosy programmes after achieving the target. However, it is becoming more evident day by day that international donor agencies have started reducing funding and manpower in NGO sectors and started diverting funds for TB programmes. This hasty decision on the part of donor agencies is likely to affect leprosy elimination activities and related research activities.

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## Book Reviews

### **An Englishwoman in Japan: Hannah Riddell, pioneer of leprosy work in Kumamoto, Kyushu, Japan, 1894–1950. Julia Boyd**

Rutland, Vermont and Tokyo, Japan. 1996. 215 pp

This is a remarkable book (hardback), describing the life and endeavours of an indomitable lady who established one of the first modern leprosaria in Japan in the year 1894. As the wife of the British Ambassador in Japan, 1992–96, the author, Julia Boyd, was invited to attend the inauguration of the Riddell-Weight Memorial Society of Kumamoto, Kyushu and this inspired her to research and publish an account of the life of Hannah Riddell, who was sent out to Japan in November 1890 under the auspices of the Church Missionary Society (CMS). Her inspiration to enter leprosy work derived from a visit to the Honmyoji temple in Kumamoto, where she encountered victims of leprosy in various stages of advanced disease and degradation. The book, which is extremely well written and commendably accurate on medical aspects, traces the 50-year story of a highly motivated and determined English lady to built and run a leprosarium in a foreign land, quite often in the face of opposition and criticism from the authorities. The hospital complex included a laboratory, to which Michitaro Uchida was appointed as first director, ‘... having been interviewed for the job by Hannah at the Imperial Hotel in Tokyo.’ Although somewhat expensive, this biography contains a wealth of information on many aspects of leprosy work in Japan in the period leading up to the Second World War, together with 57 black and white photographs, many of them of historical importance and almost certainly not published before. Number 53, on the closure of Hannah Riddell’s leprosarium at Kaishun, is particularly poignant; it shows workers dressed in protective clothing, including gum boots, masks and gloves, as they supervise the removal of patients’ furniture and personal belongings on 3rd February, 1941.

*Charles E. Tuttle*

### **Mycobacteria and human disease. John M. Grange**

Arnold, London, UK, 1996. 230 pp

From the mid-1980s, many countries identified a disturbing increase in the number of TB patients from within their own communities. WHO declared tuberculosis a global emergency. Funding for research and public health expanded and this was associated with a concomitant increase in the number of publications including many books. Simultaneously, there was an increase in attention paid to leprosy and the goal of achieving its elimination was announced as a foreseeable reality. In addition, with the advent of AIDS, atypical mycobacteria, especially *Mycobacterium avium intracellulare*, were recognized as important human pathogens. Therefore, it is surprising that so little attention has been paid to the ‘collective’—the mycobacteria themselves. No doubt, editors and publishers of TB books would argue that inclusion of other mycobacteria would dilute the ‘biggest of all infectious killers’. So why collect together this seemingly mixed bag of mycobacteria? At one level, this could be seen as a result of



microbiological and histological categorization and, in the case of this book, reflect the author's background. From a clinical perspective, the common mycobacterial diseases are a disparate collection, affecting a variety of different systems, each with its own unique natural history. However, this 'mixed bag' has more in common than its genetic ancestry alone, and a variety of useful scientific and clinical questions can be asked when comparing mycobacteria with each other. For example, are there immunological similarities between leprosy type 1 (reversal) reactions and the paradoxical worsening of clinical features occasionally seen following the commencement of anti-tuberculous treatment? Is it likely that genetic predisposition to atypical mycobacteria seen in certain Maltese families applies to its phylogenetic cousins? Is intracellular processing of mycobacterial antigen down an MHC class I pathway present for all species (with induction of CD8+ T cells) or is this limited to only some pathogenic mycobacteria.

Whether you feel that this second edition of *Mycobacteria and human disease* provokes such a debate, is not critical. What this book certainly achieves, is to remind us of the relatedness of these diseases as well as their distinctions. In its entirety, it presents an excellent overview of many aspects of mycobacteria. It fills gaps which have been previously skimmed over by equivalent texts. The author's practical and theoretical expertise is evident and, as a single author book, it offers a coherent balance of subject matter and style. The subject matter is very comprehensive considering its relatively short 230 pages. It deals with historical background, microbiology, genetics, immunology and immunosuppression, vaccines, public health and clinical disease. Particularly good are the sections covering mycobacteria cell structure, a description and classification of important species, diagnostic issues and the epidemiology/disease control section. However, single author textbooks have weaknesses. The immunology section seemed the least strong. The author concentrated on issues relating to delayed-type hypersensitivity and, although this is of interest, it represents only one aspect of mycobacterial immunity. Other issues concerning the complexities of cellular immunity and vaccine design were either missed out or alluded to very briefly. Also, the sections covering the clinical presentation, diagnosis and management of tuberculosis did not have the feel as if they had been written from a practical perspective, but came across as a catalogue of disease presentations. Despite the inevitable shortcomings of single authorship, this 'monotherapy' should be taken, as the benefits easily outweigh the adverse effects!

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## Teaching Materials and Services

### LEPRA/TM Ophthalmic Course, Karigiri, India 1998

The 13th annual 5-day ophthalmic teaching module was held at the Schieffelin Leprosy Research and Training Centre, Karigiri from the 2nd to 7th March 1998. The course, which was sponsored jointly by LEPRA through the Barclays Bank/English Speaking Union International Training Scheme and The Leprosy Mission, was designed to give instruction to leprologists on the detection, prevention and management of the ocular complications of leprosy by means of a series of lectures and clinical and surgical demonstrations, augmented by videos and a field trip.

Teaching included formal didactic presentations on the basic anatomy, physiology and pathology of the eye with a special emphasis on leprosy: in addition, there were lectures on the pathogenesis and treatment of corneal ulcers, rehabilitation, community ophthalmology and global aspects of blindness in the disease.

A preference was given this year to clinical demonstrations and discussions dealing with important aspects of ocular leprosy such as the diagnosis and management of lagophthalmos, intra-ocular inflammation and infiltrative lesions, and 'hands on' teaching methods were employed more than in previous years.

The course was attended by seven sponsored participants working in India, and was organized by Dr Ebenezer Daniel of Karigiri, with the assistance of members of the Staff of the Centre. Mr Timothy ffytche from St Thomas's Hospital, London and Dr Kirsteen Thompson from Purulia, West Bengal were invited members of the Faculty.

The Director of Karigiri, Dr P. S. S. Sundar Rao, is to be thanked for his continued support for this important and popular contribution to teaching.

### Research synthesis: its importance in developing countries

The following article by Helga Patrikios, Medical Librarian, University of Zimbabwe, appeared in volume 8, number 1, April 1997 of *Liaison*, published by WHO.

As potential users and as people working with health information, we all need to believe that the decisions made about the health care dealt out to us are based on the best available scientific evidence. Yet, most doctors agree on one thing: that the current volume of medical information is unmanageable; they report that they do not know about important advances, feel overwhelmed by new scientific information, are not good at finding new information and do not know how to evaluate it when it is found. Many of them base their decisions on their own opinions or those of colleagues who are no more knowledgeable than they. One study showed that practitioners often 'don't know what they don't know'. Richard Smith, the Editor of the *British Medical Journal*, reporting on these studies, summarised the situation this way: ... 'experienced doctors use about two million pieces of information to manage their patients' ... Unfortunately some of the information in doctor's heads is out of date and wrong, new information may not have penetrated ...' (Smith R. What clinical information do doctors need? *BMJ* 1996; 313: 1062–8).

That's why the concept of research synthesis may be the most significant trend for medical librarians since information technology (PCs, CD-ROM, e-mail and all that) came into our lives. The most surprising thing about research synthesis is that its principles were not applied to medical practice long since.

Research synthesis is the relatively recent response to a decades-old and rapidly worsening problem for clinicians and policy makers: how to manage a biomedical knowledge base that is presently doubling itself every 19 years (and in the case of HIV/AIDS every 22 months.) It is now widely accepted that reviews of research based on studies using randomized controlled trials are what is needed; these trials are generally agreed to be the best research method on which to base decisions. In effect, doctors have now progressed beyond the realization that they cannot cope with the growth in the literature; the Cochrane Collaboration and the systematic reviews of the literature being made in key areas of medical practice are acknowledgements of the need for rational solutions to the problem of information management.

Inevitably as research synthesis gains ground in continuing medical education and medical school curricula, criticisms of the concept are raised. Controversies are arising around reliability of the meta-analyses of pooled clinical trials of similar design; there is evidence that they are not always reliable. (A more fundamental question is also raised: whether EBM can be applied in a *population-health* ethic of efficiency, or whether it will be limited to an *individual-patient* ethic of effectiveness). At this point, the usefulness of research synthesis in poorer countries where population-health is the governing criterion may be questioned. The question should perhaps be reversed: why should every possible effort *not* be made to bring the benefits of research synthesis to the poorer countries? There are even fewer resources to waste on interventions that are not effective. Problems of accessing, evaluating and managing information are even more acute in situations where the pressure on health services is acute and libraries are impoverished. In such context, syntheses and summaries of critical information are essential. How systematic reviews are applied in making decisions on cost-effective interventions is a matter for national policy makers, who ought to have access to the best evidence available.

Many groups in the Cochrane Collaboration are publishing relevant reviews, including the Infectious Diseases Group, the Acute Respiratory Group and the Pregnancy and Childbirth Group. Cochrane Parasitic Diseases Group (CPDG) reviews are due to be published soon and others of relevance to developing countries should follow. Printed digests with a regional or national focus could be an economical way to bring summaries of salient reviews to health professionals whose access to electronic sources is still limited and these are something all of us should consider, whether we are librarians, practitioners or researchers.

The cooperation of medical schools, ministries of health, health information providers and medical librarians should eventually enable health professionals and students everywhere to apply the principles of research synthesis to their work—using whichever format or media are found to be appropriate, affordable and user-friendly. (At this Medical School we've started by subscribing to the BMJ's journal *Evidence Based Medicine*, which looks like a good buy at the institutional price of £80 for 24 issues).

*Helga Patrikios, Deputy University Librarian, Medical Librarian, University of Zimbabwe, P.O. Box M.P. 45, Mount Pleasant, Harare, Zimbabwe. Tel: 263-4-791631, Fax: 795019. patrikios@healthnet.zw (Internet) 5:7211/2.3065 (Zimbabwe).*

## WHO International Exchange of Health Literature

The following reminder has been taken from a back number of *Liaison*: Newsletter of the WHO Office of Library & Health Literature Services.

'We wish to remind you of this exchange scheme which has been run by the WHO Library for the last thirty years; it has 216 members in 69 countries. It acts as a clearinghouse for libraries who wish to dispose of health science and related publications, including books and periodicals and those who need such items. Libraries with material they wish to give away send us a list of what they have to offer. We

duplicate this list and send it to all members. Librarians then can contact each other directly to obtain specific items on the list. The only obligation that we ask is that libraries in the developed world pay the postage for material going to developing countries. Each member can request or donate material as they wish. We do suggest that lists of books should not include out-of-date material: books should not be more than five years old and periodicals not more than ten. Last year we distributed 200 duplicate or superseded books and 2500 issues of periodicals from our own collection. If you are interested in joining the scheme please contact us at the following address: International Exchange of Health Literature, WHO Library, 1211 Geneva 27, Switzerland, and we will gladly send you further information.'

### **TALC: 'Clinical Tuberculosis' by Crofton, Horne & Miller—now available in 18 different languages**

The standard description of this book from *Teaching Aids at Low Cost* (TALC, PO Box 49, St Albans, Herts AL1 4AX, (United Kingdom) reads as follows:

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

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

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

Since publication a few years ago the English version has had an astonishing circulation worldwide, with many calls for translation. Very largely due to the vast network of contacts known to Professor David Morley in TALC, 18 translations have now been produced (or are in progress and soon to appear). The list is as follows.

#### CLINICAL TB TRANSLATORS/PUBLISHERS

<b>India</b>	CBS Publishers and Distributors 4596/1-A 11 Darya Ganj New Delhi 110002 India	<b>Turkish</b>	Dr Elif Dagli Associate Professor of Paediatrics Marmara University Altunizade 81190 Istanbul Turkey
<b>Chinese</b>	Chinese Anti-Tb Association 5 Dong-Guang Hu-Tong Beijing 10035, China	<b>Italian</b>	Dr Stephano Nardini Ospedale di Bormio e Sondala Presidio Multizonale di Riabilitazione, via Zubiani 33 23039 Sondalo, Italy

<b>French</b>	IUATLD 68 Boulevard Saint-Michel 75006 Paris France	<b>Croatian</b>	IBIS Grafika doo (Mr Kresimir Krmic) IV Ravince 25 1000 Zagreb Croatia
<b>Portuguese</b>	Editora Guanabara Koogan Tv do Ovidor 11 Rio de Janeiro RJ, Brazil 20040-040	<b>Urdu*</b>	Dr Ghazala Ansari Director/Chest Specialist Ojha Institute of Chest Diseases University Road 75270 Karachi 32 Pakistan
<b>Spanish</b>	Imprimerie CMI Zl de Mitry-Compans 25 rue Marc Seguin 77290, Mitry-Mory France	<b>Mongolia</b>	Dr G Tsogt Mongolian Anti TB Assoc PO Box 90 Ulaan Baatar-49 Mongolia
<b>Vietnamese</b>	National Institute of TB & Respiratory Diseases 120 Hoang Hoa Tham Hanoi Vietnam	<b>Russian</b>	Prof A G Khomenko Central Tuberculosis Research Inst 2 Yauzskaya Alley Moscow 107564, Russia
<b>Thai</b>	Prof Vichitbandha Faculty of Medicine Thammasat University Ransit Campus Klong-Luang Prathum-Thanse 12120 Thailand	<b>Arabic (ACML)</b>	Dr Y Al-Sharrah Assistant Secretary General Arabic Center for Medical Literature PO Box 5225 Safat 13053 Kuwait
<b>Farsi (Iran)</b>	Dr H Heidarnajad PO Box 51335-1618 Tabriz Iran	<b>Indonesian*</b>	EGC Medical Publisher PO Box Jakarta 10042 Indonesia
<b>Romanian*</b>	Dr Traian Mihaeseu EDIT-DAN Publishing Co PO Box 209-6600 Iasi, Romania	<b>Bengali*</b>	Dr Zafrullah Chowdhury Gonoshashteya Kendra (GK) PO Nayarhar via Dharmrai Dhaka, Bangladesh

\*Indicates translations in progress.

*Further information:* TALC, PO Box 49, St Albans, Herts, AL1 5TX, United Kingdom. Tel: +44(0)1727 853869 Fax: +44(0)1727 846852

### **Topics in International Health: 'CD-ROMs tackle global diseases': The Wellcome Trust, London, UK, 24 April 1998**

The Governors and Director of the Wellcome Trust (183 Euston Road, London, NW1 2BE, UK) invited over 100 people, most of them involved in tropical or third world medicine, to a meeting in London on 24 April, 1998, to celebrate the launching of an important new series of educational CD-ROMs (Compact-Disc-Read Only Memory), 'Topics in International Health'.

Those invited included representatives of the London medical schools, the schools of tropical medicine in London and Liverpool, The Wellcome Trust, The Department of Health, The National Institute for Medical Research, the Health Education Authority, the Royal Society of Medicine, the UK Cochrane Centre, the Department of International Development, The British Medical Association, the Royal College of Physicians of London, The Hospital for Tropical Diseases, INASP-Health and CAB International, together with other agencies and individuals involved in the production and distribution of appropriate health education in the UK to lesser developed countries.

Following the opening address by Dr Bridget Ogilvie, Director, the Wellcome Trust, Mr Paul Boateng, member of Parliament and Parliamentary Under-Secretary of State for Health, gave an address in which he emphasized the measures currently being taken by the Government to alleviate poverty in third world countries, whilst at the same time supporting initiatives which tackle the burden of tropical diseases.

The first four CDs on malaria, sexually transmitted diseases, trachoma and sickle cell disease, were published in April 1998. Four more, on leprosy, tuberculosis, diarrhoeal diseases and schistosomiasis, are due in the autumn of 1998 and two more, on AIDS/HIV and nutrition in 1999. The series is intended for use by medical and life science students, lecturers, health care professionals, academics and researchers in both developed and under-developed countries. It can be run on Windows 3.1, Windows 95 or Windows NT.

To disseminate these materials as widely as possible, the Wellcome trust is working with CAB International, a not-for-profit organisation with world wide publishing expertise. CABI's '*Information for Development*' programme specializes in disseminating information in developing countries.

*Further Information: Topics in International Health*, The Wellcome Trust, 183 Euston Road, London NW1 2BE. Tel: 0171 611 8888/Direct 8777. Fax 0171 611 8545/Direct: 8237. Email c.griffiths@wellcome.ac.uk or *CAB International* a) UK. Wallingford, Oxon, OX10 8DE, United Kingdom. Tel: 44 (0) 1491 832111. Fax: 44 (0) 1491 826090. Email publishing@cabi.org b) USA. 198 Madison Avenue, New York, NY10016-4314, USA. Tel: 1 212 726 6490/6491. Fax: 1 212 686 7993. Email cabi-nao@cabi.org

## **TALC, UK: Leprosy in Childhood: colour transparency teaching set with text**

The original set of 24 slides and text, issued in 1979, sold 5,681 copies to virtually all leprosy-endemic countries. A revised set sold an additional 687 between 1990 and 1998, bringing the total to 6,368. The text has now (1998) been revised and up-dated, to include recent WHO recommendations on short-course chemotherapy. Apply TALC *Teaching Aids at Low Cost*, PO Box 49, St Albans, Herts AL1 4AX, United Kingdom. Fax (0) 727 46852.

## **ALERT Training Calendar 1999**

### **January 25–February 26**

#### **Prevention and management of disabilities**

Target group: physiotherapists, occupational therapists, podiatrists as well as experienced leprosy workers involved in POD. Emphasis on both patient care (early detection of nerve deterioration, health promotion, problem solving) and programme management (POD management, home based care and rehabilitation).

### **March 8–March 19**

#### **Introduction to leprosy for physicians**

Highly recommended for the participants in the following 'Management of Combined Programmes' course who need to refresh their knowledge of clinical leprosy. The course can also be taken on its own

by physicians responsible for diagnosis, treatment and care of patients with leprosy in either a hospital or a control programme setting.

### **March 22–April 16**

#### **Management of combined leprosy and tuberculosis control programmes for physicians**

Target group: experienced physicians responsible for managing a leprosy and TB control programme at the regional level or above. Emphasis on programme management: needs analysis, action plan, implementation of activities, supervision, evaluation, management of POD. A brief review of the essentials of TB is included, but leprosy expertise is a prerequisite. Participants lacking the latter should also take the preceding ‘Introduction to leprosy’ course.

### **May 10–May 27**

#### **Essentials of leprosy and tuberculosis for administrative and programme support staff**

Target group: administrative and managerial staff without a medical background, working in leprosy and TB programmes and donor agencies. Objectives: to gain a better understanding of the two diseases, to communicate more effectively with the medical staff, and to contribute more efficiently in decision making and priority setting.

### **June 7–June 16**

#### **Leprosy for researchers and scientists**

Course aimed at scientists who are familiar with leprosy mainly in a laboratory or other research setting or as figures on a computer screen, to show them what leprosy means for the patient, clinically as well as psychologically and socially.

### **September 13–October 22**

#### **Essentials of leprosy and tuberculosis for physicians**

Target group: physicians with limited experience in either leprosy or TB. Emphasis on clinical aspects of leprosy and TB, individual patient care and its application in the context of a combined programme, with an introduction to health promotion and managerial issues, paying special attention to POD and supervision.

### **November 8–November 19**

#### **Introduction to leprosy for senior field staff**

Highly recommended for the participants in the following ‘Management of Combined Programmes’ course who need to refresh their knowledge of clinical leprosy. The course can also be taken on its own.

### **November 22–December 17**

#### **Management of combined leprosy and tuberculosis control programmes for senior field staff**

Target group: experienced nurses, paramedical workers or supervisors responsible for leprosy and TB control at the district (or equivalent) level. Emphasis on planning, implementation, supervision and evaluation of control activities, with special attention for POD, health promotion and support functions. A brief review of the essentials of TB is included, but leprosy expertise is a prerequisite. Participants lacking the latter should also take the preceding ‘Introduction to leprosy’ course.

For further information, please contact: ALERT Training Division, P.O. Box 165, Addis Ababa, Ethiopia. Tel: 251-1-711524 or 251-1-712792, Fax: 251-1-711199 or 251-1-711390, Email: ahri@telecom.net.et

## News and Notes

### **ActionAid Disability News: the Newsletter of the Disability Division of ActionAid in India**

*ActionAid Disability News* is a bi-annual newsletter of the *Disability Division of ActionAid* in India, PO Box 5406, No 3, Rest House Road, Bangalore, 560 001. The Newsletter is meant for private circulation only, for planners, administrators, health professionals, funding organizations and implementing organizations involved in disability and rehabilitation programmes. Its main emphasis is on articles related to policy development, concept clarification, development of methodology in areas of service delivery, training of manpower and programme evaluation and the development of technology related to rehabilitation. Action Aid is interested in exchanging copies of the Newsletter on a reciprocal basis with other rehabilitation publications and in gathering information on programmes and research findings related to disability and rehabilitation. Apart from a series of valuable original articles in the latest issue (Vol 9, No 1, 1998), the pages are full of 'networking' information on meetings, seminars, workshops, publications and training courses.

### **Researchers create designer antibody**

The following appeared in the *British Medical Journal* vol 316, 7/3/98, page 726:

Immunologists have designed an antibody that can specifically suppress just one section of the immune system, rather than having to knock out the whole system. This antibody could revolutionize the lives of people who undergo organ transplantation because the T lymphocytes that cause organ rejection could be selectively 'turned off' by it, leaving the rest of the immune system intact to fight off rejection.

Patients who receive bone marrow and other organs from donors run the risk of organ rejection. To prevent this happening, they are conventionally given chemotherapeutic agents, such as cyclosporin, which effectively suppresses the entire immune system. Although this means that the foreign tissue which the patients have received is not immediately rejected, it also means that they are left with few defences against invasive infections or other diseases. They are often critically immunocompromised.

Now Yan Qi and Uwe Staerz from the National Jewish Medical and Research Centre in Colorado have created a hybrid antibody that will allow helper T cells to recognize a foreign organ but not allow these T cells to become activated and cause organ rejection (*Nature Biotechnology* 1998;16:271–6). Conventional antibody molecules have two identical arms that bind them to a specific target. This designer hybrid antibody has one binding arm, which is directed to cells on the transplanted organ and a second arm, which interferes with the activation of specific helper T cells. The result is that these T cells recognize the organ as foreign but are prevented from being activated. No other T cells are affected.

The binding arm of the hybrid antibody binds to all foreign cells with a specific class II HLA type. Dr Staerz calculates that to cover virtually all donor and recipient combinations of HLA types it will be necessary to make just four or five antibodies. As a result of selective immunosuppression, he believes that 95% of all transplants will need no additional minor antigen matching. A further advantage may be conferred by administering the hybrid antibody: patients may only have to take this treatment in the



short term. Dr Staerz believes that the T cells which are responsible for organ rejection may eventually be destroyed by the antibody activity, thus preventing organ rejection in the future.

So far these hybrid antibodies have been shown to work in tissue culture. Dr Staerz and his colleagues will be testing them in animal models within 6 months, and if all goes well they expect to start clinical trials within 2 years.

### **WHO Committee Identifies Constraints That Stall Progress Against TB. Statement by Ad Hoc Committee on the Global TB Epidemic, London 17–19 March 1998**

The members of the Ad Hoc Committee convened by the World Health Organization call on world leaders to give their urgent attention to the global tuberculosis epidemic. Excellent progress against the global TB epidemic in nearly 100 countries is being overshadowed by the stalled or slow progress in many of the 22 countries which account for the vast majority of the world's TB cases. The Committee notes with deep concern that even where progress has been good, questions of sustainability and expansion pose risks for the near future in places such as China and Bangladesh. Global targets cannot now be met.

Intensified technical efforts will not by themselves bring about the acceleration and expansion needed. This Committee has identified six principle constraints choking action by health authorities. These are financial shortages, human resource problems, organizational factors, lack of a secure supply of quality anti-TB drugs, and public information gaps about TB's danger. The most fundamental constraint is the lack of political will to develop and sustain effective TB programmes.

It is of course primarily the responsibility of the political and health leaders of the countries faced with the epidemic to execute an effective response. But, it is transparently in the world's public interest that the global community help fight the epidemic wherever it exists. Since progress has been too slow overall and is stalled in some key countries, extraordinary measures now are needed to reverse the insufficient political will which underpins the other constraints. The keys to effective global action to do this are in the hands of a small number of political, legislative, financial and health leaders in the endemic countries and in the developed nations and the global institutions.

The Committee believes that many of the world's leaders are unaware of the dimensions and costs of the TB epidemic and the urgency of controlling it. It also believes that most citizens in the affected countries are unaware of the risks they face and the fact that these can be eliminated by concerted use of the DOTS strategy. The world cannot be protected from TB as long as countries with a high burden of TB do not make progress. Because of HIV's impact on TB and emerging drug resistant forms of TB, the dangers of national and global inaction are increasing sharply. This committee has concluded that the insufficient political will to control TB is the greatest single constraint to progress. Political action to make and keep tuberculosis control as a true social and developmental priority would allow progress to be made against the financial, human resource, organizational, drug supply and information constraints. The committee calls on heads of state, parliamentary leaders, and finance, planning and health ministers to exercise their pivotal roles.

The global institutions for a coordinated initiative to support committed national leaders exist and must also exercise their mandate. A coordinated partnership of the WHO, the World Bank, bilateral development assistance agencies, the IUATLD and other NGOs and the global research community is needed urgently. These institutions can help sustain the environment to encourage political will. Then they can methodically and persistently alleviate the other identified constraints through policy and technical collaboration with the endemic countries and by financing and supporting the DOTS strategy, including the research to permit its wider and easier use and to develop new tools.

The Committee will finalize its full report for discussion with WHO leaders within a few days.

*Further enquiries:* Global Tuberculosis Programme, WHO, 1211 Geneva 27, Switzerland.

## India approves leprosy vaccine

The following appeared in the *British Medical Journal*, volume 316, 7/2/98, page 414:

A vaccine against leprosy has been approved by India's drug control agency and is to be incorporated into the national eradication programme. The vaccine is designed to be used as an adjunct to standard multidrug therapy to accelerate healing and reduce the duration and cost of the treatment.

The vaccine, developed at the National Institute of Immunology in New Delhi, is said to be the first in the world that stimulates the immune system to kill *Mycobacterium leprae*. The vaccine, administered intradermally, is prepared from a killed non-pathogenic strain of *Mycobacterium*, first isolated in the mid-1970s from the sputum of a patient with tuberculosis in Madras.

'Patients who receive the vaccine and standard anti-leprosy multidrug treatment show faster clinical improvement and more rapid clearance of bacteria than those who receive only drugs,' said Dr Rama Mukherjee, a senior scientist at the Institute. Whereas multidrug therapy using rifampicin and two other drugs takes 12–24 months, the vaccine will help to reduce duration of treatment by at least 6 months in the most severe cases, Dr Mukherjee said.

'We expect this vaccine to provide a big boost to the leprosy eradication programme,' said Dr Manju Sharma, secretary of India's department of biotechnology, which invested about 20 million rupees (£300,000; \$480,000) in the project. Leprosy is prevalent across Asia, Africa, and Latin America, but India accounts for 60% of the global pool of patients with leprosy, estimated to be about one million in 1996. A fifth of patients are below the age of 18.

The vaccine is based on the concept of 'cross reacting antigens,' in which the killed *Mycobacterium* strain is used to stimulate the immune system into mounting an attack on *M. leprae*. 'This is possible because the two bacilli have cross reacting antigens,' said Dr Mukherjee. The first commercial batch is expected to be released by June 1998 and will be sold in India at six rupees (10p) a dose.

Health ministry officials, however, have expressed reservations about the impact of the vaccine in the leprosy eradication programme. 'We don't see any real advantage of using this adjunct. Patients who are on standard multidrug therapy are not expected to actually feel any benefit from the faster clearance of the bacteria brought about by the vaccine. Drug treatment alone does lead to complete elimination of bacteria, although the process may be slower,' said a senior official.

Others argue that the vaccine has been known to cure the disease and clear bacteria within 6 months in some patients. 'It will also help prevent reactivation of the disease in the most severe cases,' said Gursaran Talwar, former director of the National Institute of Immunology. India is nowhere near eradicating leprosy with the current treatments available. Last year the health ministry detected 400,000 new cases.

Institute scientists say that the immunoprophylactic role of the vaccine is also under investigation. Over the past 8 years, nearly 23,000 health household contacts of patients with leprosy have received the vaccine, but the results of this study are not expected for another 3 years because of the long gestation period of the leprosy bacillus.

## Global research funding for AIDS, malaria and tuberculosis

Writing in the latest issue of *EDIT*, *The University of Edinburgh Magazine*, issue 14, Summer 1998, Bryan Christie describes the present world situation with regard to malaria and the prospects for the development of a vaccine. The final paragraphs include the following: 'Figures which compare global research funding against the annual death toll from particular diseases highlight the plight: HIV/AIDS gets \$3274 per death: asthma \$789: malaria \$65 and tuberculosis \$13.' [*EDIT*, The University of Edinburgh Centre, 7–11 Nicolson Street, Edinburgh].

### Scotland as a focus of world electronics industry

The following appeared in the latest edition of EDIT, The University of Edinburgh Magazine, Issue 14, Summer 1998, page 4:

Working closely with Scottish Enterprise, Edinburgh, Glasgow, Heriot Watt and Strathclyde Universities have launched the world's first Institute for System Level Integration. The Institute is being developed through a unique collaboration of the four universities working with an international panel of experts and Scottish Enterprise to create a focus for both research and teaching in this emerging discipline. The Institute, which will make Scotland a world centre for next generation semiconductor research and design, is to be based in a new Design Complex being built at Livingston near Edinburgh which will include the creation of 1,895 highly skilled new jobs by Cadence Design Systems of San Jose, California, the world's leading electronic design automation software and design services company. The Institute is also committed to deliver professional development modules from 1998 and, as soon as possible thereafter, a full-length MSc course targeted at students with a first degree in Electrical Engineering or Computer Science.

Cadence have also announced a University Scholarship programme which will award eight prizes to the top graduates in both Computer Science and Electrical Engineering at Edinburgh, Glasgow, Heriot-Watt and Strathclyde Universities. The prizes will be given either as £1,000 in cash or a 1-year sponsorship of an approved Masters degree in System Level Integration at any of the four universities.

### INASP: International Network for the Availability of Scientific Publications

The following items are taken from the latest *INASP Directory* (1997/1998):

#### Commonwealth Secretariat

Commonwealth Secretariat  
Health Department  
Marlborough House  
Pall Mall  
London  
SW1Y 5HX  
UNITED KINGDOM

*Contact:* Dr Helen Bicken

*Telephone:* (44 171) 747 6291 *Fax:* (44 171) 747 6287

The Commonwealth Secretariat, through its Health Department, is involved in technical cooperation. Proposals include:

- development of linkages between health institutions in Commonwealth countries for interchange of personnel, information and training opportunities;
- development of training materials in response to needs expressed at Commonwealth Health Ministers Meetings;
- gathering of information on key health issues from Commonwealth countries and making this available, production of reports on expert group meetings, workshops or specific projects and sharing these with Commonwealth countries.

### **African Medical and Research Foundation (AMREF)**

African Medical and Research Foundation  
Publishing Department  
Wilson Airport  
PO Box 30125  
Nairobi  
KENYA

*Contact:* Alice Nabwera

*Telephone:* (254 2) 501301/3 *Fax:* (254 2) 602495 *E-mail:* 62057276@eln.attmail.com

The African Medical and Research Foundation (AMREF) is an African non-profitmaking non-governmental organization committed to health development and aims to produce health learning materials that are more appropriate and cheaper than the equivalents imported from other countries. The Department has some 40 health manuals on its list and is constantly developing new materials or revising its popular titles.

As a Kenyan-based professional publishing body with its own printshop and an established distribution system, the AMREF Publishing Department is also well placed to produce printed materials for ministries of health, health-related NGOs and international organizations working in the region as well as serving the needs of its own programmes.

An alternative contact is Caroline Agola.

### **APNET (African Publishers Network)**

APNET (African Publishers Network)  
PO Box 3773  
11th Floor Megawatt House  
44 Samora Machel Ave  
Harare  
ZIMBABWE

*Contact:* Gillian Nyambura

*Telephone:* (263 4) 705105 *Fax:* (263 4) 705106 *E-mail:* apnet@mango.zw

The African Publishers Network was established at the Harare conference of the African Publishing Institute on 17th February 1992.

The Network believes that the most viable way in which Africa can solve the current book crisis is through the long term expansion of indigenous publishing activities. This refers to all manufacturing of books in Africa, from research, scholarship and authorship through to printing, marketing and distribution.

The detailed objectives of APNET are:

to address urgently the need for effective communication between African publishers, and between countries and regions; on developments affecting or likely to affect African publishing as a whole, through the medium of a newsletter;

to set up an information-gathering system to bring together information on developments affecting African publishing from its many diverse sources;

to analyse critically trends in African publishing and, where necessary, to produce policy documents reflecting the views and position of indigenous African publishers;

to encourage and assist inter-African trading in books and joint ventures between African publishers;

to assist in the establishment of national publishers associations throughout Africa;  
 to work towards an association of African publishers as a body genuinely representative of indigenous African publishing throughout the continent; and  
 to expand and improve the availability and scope of training for publishing and book distribution to Africa.

The project will in practice be based on six inter-related activities:

- the creation of a resource centre of documentary material on African publishing which will include information from the major book periodicals, policy statements and conference documents, and essays and articles on African books;
- the publication and distribution of a trilingual newsletter (*The African Publishing Review*) six times per year, the basis of which shall be country reports of major publishing developments, regional reports, special features on publishing conditions within a country, and other news affecting or likely to affect African publishers;
- the research and publication of occasional documents reflecting policy thinking on indigenous African publishing;
- inter-regional contact and networking involving travel by African publishers to attend important events in Africa, to collect information, to hold discussions, to brief publishers on the activities of APNET, to assist in inter-African book trading and to assist in the establishment of national publishers associations;
- representation: to ensure indigenous African publishers' participation through APNET at major international conferences and otherwise to meet important multi-lateral agencies involved in support for African publishing;
- to engage in the development of an African Publishing Institute for the expansion, improvement and coordination of training facilities available to African publishing.

APNET is both representative of indigenous publishing in Africa and is a democratic organization. The priorities for programmes and activities are determined through meetings and discussions at different levels (e.g. national publishers association to APNET General Council to APNET Administrative Committee for implementation) and through extensive consultation. The single most important factor determining the type of activities APNET supports is how this affects the long-term viability and sustainability of development in indigenous publishing.

APNET is committed above all to the idea that long-term book provision in Africa can only be sustained through the expansion and development of indigenous publishing.

The APNET contacts are: Gillian Nyambura and Tainie Mundondo (APNET Secretariat Committee, Harare). Victor Nwankwo (Nigeria), Henry Chakava (Kenya), Hamidou Konate (Mali), Samuel Matola (Mozambique) and Jane Katjavivi (Zimbabwe) are the regional representatives for West, East, francophone, lusophone and Southern Africa.

## ExtraMED

ExtraMED  
 Informania Ltd  
 PO Box 40  
 Petersfield, Hants GU32 2YH  
 UNITED KINGDOM

Contact: Diana Zielinski

Telephone: (44 1730) 301297 Fax: (44 1730) 265398

E-mail: 100060.172@compuserve.com

*ExtraMED* originated from a project of the World Health Organization. It consists of the publication in CD-ROM format of copies of health and biomedical journals, containing all the text and illustrations of the original journal articles. The text is in page images—printouts look like photocopies of the original articles, catalogued and searchable by keyboard.

Taking its name from the fact that it comprises journals that are 'extra' to *MEDLINE*, *ExtraMED* focuses on journals that are largely excluded from the international indexes. The *ExtraMED* Consortium of Journals now comprises over 290 biomedical journals throughout the world, selected through WHO's various Index Medicus projects. *ExtraMED* is designed to serve the purposes of promoting the literature of developing countries, while subsidising its production and development through subscription revenue. At the same time, it provides a powerful new research and diagnostic tool.

The journals in *ExtraMED* are outside the coverage of the MEDLINE services and are mostly from developing countries. They include many topics that MEDLINE journals don't cover adequately, e.g. tropical diseases, traditional medicine and biodiversity, cholera and other waste-borne diseases, etc. Printed copies of the articles can be made from the disc.

The main advantage of *ExtraMED* is that it gives the most important medical journals of the non-MEDLINE world in one source. It should be of great interest to developing countries in view of the subject matter and coverage of the journals contained in the disc.

The CD-ROM is published every month and contains the equivalent of 8000 pages.

## **Tropical Medicine Resource (TMR)**

Tropical Medicine Resource  
Wellcome Centre for Medical Science  
The Wellcome Trust  
210 Euston Road  
London NW1 2BE  
UNITED KINGDOM

*Contact:* Head, Tropical Medicine Resource

*Telephone:* (44 171) 611 8603 *Fax:* (44 171) 611 8270 *E-mail:* tmr@wellcome.ac.uk

The Tropical Medicine Resource is funded by The Wellcome Trust. It is targeted at early postgraduate or senior undergraduate medical and life sciences students and their tutors, based in centres of higher learning throughout the world. It has two component parts, both delivered on CD-ROM:

the Visual Archive is a searchable, electronic database of images illustrating a wide range of aspects of tropical medicine from clinical features to the environment. Each image presented is catalogued, described and copyright-cleared. A loan service for obtaining copies of the images is planned but is not yet fully operational;

the Computer-Interactive Tutorials provide an introduction to key topics in tropical medicine or a means of quick revision. They are highly visual learning materials that allow user interaction and provide learning objectives and self-assessment

The materials are designed for ease of use but support materials are provided as a backup. These include on-screen help, an on-screen glossary of medical and scientific terms, a printed program manual, a printed glossary and quick reference screen guides. The materials are also designed to be reliable, and all information presented is checked by external subject experts.

At present the TMR CD-ROM is available for appraisal by any centre of higher education that has the appropriate hardware and is willing to agree to fulfil the appraisal requirements.

TMR CD-ROM currently covers the following six topics: anaemia in pregnancy, malaria, schistosomiasis, sexually transmitted diseases, sickle cell disorder and trachoma. The TMR is working to extend the resource to cover additional topics; immediate priorities are tuberculosis and diarrhoeal diseases.

### **Instituto de Investigação Científica Tropical (IICT)**

Instituto de Investigação Científica Tropical  
Rua da Junqueira 86  
1300 Lisbon  
PORTUGAL

*Contact:* Joaquim Alberto da Cruz e Silva, President

*Telephone:* (351 1) 364 5071 *Fax:* (351 1) 363 1460

The Institute for Tropical Sciences Research (IICT) dates from 1883 with the aim of developing scientific research in tropics. In order to achieve its objectives IICT has a number of programmes:

- to coordinate activities resulting from scientific relations between Portugal and tropical countries (mainly those countries with the Portuguese language, but also 40 other countries);
- to plan scientific and technical cooperation activities with these countries;
- to implement collaborative scientific assistance and exchange programmes as well as training programmes through scholarship schemes;
- to support university teaching and research in areas of its scientific activities.

IICT has a staff of 426 of whom 157 are research fellows and collaborating university professors. It has 23 specialized research centres which cover areas such as: Agrarian Sciences, Geographic Engineers, Biological Sciences, Earth Sciences, Historical, Economic and Sociological Sciences, and Ethnological and Ethno-Museological Sciences. It also possesses a substantial Documentation and Information Centre.

IICT is Portugal's major scientific publisher, having published more than 8000 scientific works, over 1600 books and review numbers, and nearly 1600 geographical, hydrographical and geological maps. The Institute has a large exchange programme with other institutions working in similar areas.

IICT is a member of the ECART (European Consortium for Agricultural Research in the Tropics) jointly with CIRAD (France), NRI (UK), KIT (Netherlands), ATSAF and GTZ (Germany). Together, these organizations represent a European capacity of over 2500 professional staff and several research centres in the tropical regions.

### **Book Aid International**

Book Aid International  
39–41 Coldharbour Lane  
London  
SE5 9NR  
UNITED KINGDOM

*Contact:* Sara Harrity, Director

*Telephone:* (44 171) 733 3577 *Fax:* (44 171) 978 8006 *E-mail:* rls@gn.apc.org

Book Aid International, formerly Ranfurly Library Service, was founded in 1954 and is a voluntary

organization with 30 members of staff supported by many volunteers. Its core programme meets requests for books from developing countries at all levels and in all subjects by supplying new and used donated books.

The rest of the books are purchased to fill gaps in donated supplies. Book Aid International buys essential titles in very high demand and low supply, for example atlases and dictionaries for learners of English as a second language. It also buys books for children and adults published within Africa to strengthen the cultural relevance of its ongoing programmes and support the development of local publishing.

Currently, Book Aid International sends over half a million books each year to support literacy, education, training and publishing in over 60 developing countries. Eighty percent of the books support projects in 13 countries in sub-Saharan Africa.

The organization works in partnership with organizations which are able to distribute books most effectively in their country. All requests are assessed according to Book Aid International's recently revised criteria for book aid. Books are sent via an established distributor, often the public library service in the country concerned, which then allocates the books according to local needs and priorities. Or they are sent direct to the requesting institution.

An alternative contact is David Membrey, the Deputy Director.

## **World Health Organization (WHO)**

World Health Organization  
Office of Library and Health Literature Services  
20 Avenue Appia  
CH-1211 Geneva 27  
SWITZERLAND

*Contact:* Yvonne Grandbois, Acting Chief

Telephone: (41 22) 791 2071 *Fax:* (41 22) 791 4150 *E-mail:* hlt@who.ch

*Web site:* <http://www.who.ch>

The Office of Library and Health Literature Services, also known as the WHO Library, operates an international exchange of health science books and periodicals. Membership in the exchange simply entails sending in the name of the library to the WHO Library. Participating libraries send their lists of offers and needs to the WHO Library which duplicates the lists and sends them to the member libraries. These libraries then contact each other to request specific items. Libraries in the developed world are asked to pay the postage for material going to the developing world.

WHO Library's products *WHODOC*, *Liaison* and the *Library Digest for Africa* are available on the WHO gopher on the internet, or in printed form for libraries without Internet access.

A project with which WHO is associated is the *African Index Medicus* (AIM) launched in 1993. This is produced under the leadership of the Association for Health Information and Libraries in Africa (AHILA). AIM seeks to improve access to the contents of African health and biomedical journals. Requests for more information on the AIM Project should be addressed to Lucilda Hunter at the Library and Documentation Centre, WHO Regional Officer for Africa, PO Box 6, Brazzaville, CONGO (*tel:* (242) 839111; *fax:* (242) 839400).

A new project entitled *Les Bibliothèques bleues* (Blue trunk libraries) is being launched for francophone Africa. It is a collection of books and manuals suitable for district health centres.

## **International Union of Pharmacology (IUPHAR)**

International Union of Pharmacology  
Department of Pharmacology  
Faculty of Medicine, University of Montreal



Case Postale 6128  
Montreal, Quebec H3C 3J7  
CANADA

*Contact:* Prof Paul du Souich, Secretary, Clinical Pharmacology Division

*Telephone:* (1 514) 343 6335 *Fax:* (1 514) 343 2204

*E-mail* dusovic@ere.umontreal.ca

*Web-site:* <http://iuphar.pharmacology.unimelb.edu.au/>

The object of the International Union of Pharmacology (IUPHAR) is to foster international cooperation in pharmacology by:

- promoting cooperation between societies representing pharmacology and related disciplines throughout the world;
- sponsoring international and regional congresses and meetings and helping in their organization by establishing advisory committees;
- encouraging international cooperation and free exchange of scientists and of ideas in research;
- acting as a body through which pharmacologists can participate with scientists from other disciplines in international activities

IUPHAR has provided, since 1980, monthly copies of *Trends in Pharmacological Sciences* to various developing country institutions, upon the recommendation of its Executive Officers. The 1994 value of these subscriptions was estimated at about £4500. A survey of recipients is made every 3 years.

An alternative contact is Professor W C Bowman, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XU, UK.

### **Commonwealth Pharmaceutical Association (CPA)**

Commonwealth Pharmaceutical Association  
1 Lambeth High Street  
London  
SE1 7JN  
UNITED KINGDOM

*Contact:* Philip E. Green, Secretary

*Telephone:* (44 171) 735 9141 *Fax:* (44 171) 735 7629

The Commonwealth Pharmaceutical Association (CPA) makes recent issues of the *British National Formulary* available on an annual basis to pharmacy departments, pharmacists and other health professionals. The CPA arranges collection within the UK; Book Aid International provides shipment to its distributors in developing countries in the Commonwealth and the local CPA representative is responsible for collection of the books and decides upon the nature and extent of distribution.

### **American Society of Clinical Pharmacology and Therapeutics (ASCPT)**

American Society of Clinical Pharmacology and Therapeutics  
c/o Dept of Pharmacology  
Cornell University Medical College  
1300 York Avenue—Box 70  
New York  
NY 10021  
U.S.A.

*Contact:* June Reidenberg, Managing Editor

The American Society of Clinical Pharmacology and Therapeutics (ASCPT) subsidises jointly with the publishers of its journal *Clinical Pharmacology and Therapeutics*, Mosby-Year Book Inc., a number of gift subscriptions to *Clinical Pharmacology and Therapeutics* for selected libraries in Third World medical schools and Ministries of Health in 27 countries. It has provided advice to other US medical societies in developing their own programmes.

*Further information:* INASP, 27 Park End Street, Oxford OX1 1MU, United Kingdom.

### **Partners: Magazine for Paramedical Workers: The Leprosy Mission International**

*The Leprosy Mission International* (TLMI) has recently issued Numbers 31, 32 and 33 (volumes 1 and 2, 1996/97, focussing on eye care in leprosy, with important contributions from experts in ophthalmology in the Netherlands, India, Singapore and the United Kingdom. They include numerous colour plates and diagrams on diagnosis, differential diagnosis and treatment. Recommended teaching and learning material includes the following:

*Community Eye Health* is an excellent magazine dealing with general issues about eye health. For more information contact

Journal of Community Eye Health,  
International Centre for Eye Health,  
Institute of Ophthalmology,  
11–43 Bath Street,  
LONDON,  
EC1V 9EL, UK

Dr Paul Courtright's book, *Prevention of Blindness in Leprosy*: is also available from the above address.

*Essentials of Leprosy*: Dr Leo Yoder: New edition of this informative booklet now includes colour plates – original author, Dr Pearson. New eye chapter by Dr Elspeth Zijp. *English*.

*Guide to ocular leprosy for health workers*: Paul Courtright and Susan Lewallen. Simple, effective measures to prevent blindness in leprosy patients. *English*.

*Training health workers in Ocular leprosy*: Paul Courtright and Lewallen. For trainers of health workers using the Guide to ocular leprosy. *English*.

*Care of the eye in Hansen's Disease*: Dr Margaret Brand: Book for Ophthalmologist treating leprosy patients. *English*.

*Prevention of Disabilities – Guidelines for leprosy control programmes*: ILEP 1995. Eye contributions by Margreet Hogeweg. *English and French*.

*PLA Notes* (Notes on Participatory Learning and Action) is published three times each year in February, June and October. PLA Notes enables practitioners of participatory methodologies from around the world to share their field experiences, conceptual reflections and methodological innovations. To receive further information about PLA Notes subscriptions please contact

Hilary Pickford,  
IIED  
3 Endsleigh Street  
London  
WC1H 0DD, UK

Tel +44 (0)171 388 2117  
Fax +44 (0) 171 388 2826  
email sustag@iied.org

*The Nepal Participatory Action Network* came into being in January 1995. NEPAN exists to promote and advocate the need, importance and skills of participation and skills of participation for empowering the people who are the subject of development.

Nepan has a resource center which can be used by members and non-members. For more information contact Kamal Phuyal, Co-ordinator, Nepal Participatory Action Network, GPO Box 890, Batule Ghar, Dilli Bazar, Kathmandu, Nepal.

Tel +977 1 421617  
Fax +977 1 419718  
email nepan@mos.com.np

TALC (Teaching Aids at Low Cost) has a new catalogue out for 1998, which includes new and revised materials for 1998. To obtain a catalogue and price list please write to

TALC  
P O Box 49  
St Albans  
Hertfordshire  
AL1 4AX  
UK  
Tel: +44 (0) 17278 53869  
Fax: +44 (0) 17278 46852

Translations of *Partners* may be obtained by contacting the following:

For *India, Sri Lanka and Myanmar*, the address is The Leprosy Mission, CNI Bhavan, Pandit Pant Marg, New Delhi, India.

For copies of the *Bengali* edition write to: Dr Chaudhury, Grecales Training Centre, 23 Market Street, Calcutta 700 087, India.

For copies of the *Hindi* edition write to: Mr P K Roy, Leprosy Mission, CNI Bhavan, Pandit Pant Marg, New Delhi, India.

For copies of the *French* edition, Associés, write to: La Mission Evangélique Contré la Lèpre, Chemin de Réchoz, 1027 Lonay, VD Switzerland.

For copies of *Partners* in *Chinese* please write to Dr Zhao Xiding, Vice-Editor, China Leprosy Journal, 402, 87 Hengfu Road, Guangzhou, 510095, China.

*Partners* is printed and despatched in Singapore on behalf of: The Leprosy Mission S.E. Asia, 6001 Beach Road, #08-06, Golden Mile Tower, Singapore 199589.

The Leprosy Mission International, 80 Windmill Road, Brentford, Middlesex, TW8 OQH, U.K.  
E-mail: junen@tlmint.org

## **Letter to the British Medical Journal: 'Dominant gene probably caused some of the defects ascribed to thalidomide'**

Following a series of alarmist and potentially misleading articles in the lay press recently, the following letter from Professor Dick Smithells was published in the *British Medical Journal*, volume 316, 10th January 1998:

EDITOR—The Journal *Teratogenesis, Carcinogenesis and Mutagenesis* published a highly controversial paper by Huang and McBride purporting to show experimental evidence which might 'explain' second generation defects in the offspring of people accepted as having been damaged by thalidomide. Bower

asks why the journal should publish this paper alongside a devastating critique by Neubert, a member of the journal's editorial board, saying that 'the paper...contains so many inadequacies that it is impossible to draw any conclusions.

I wish to put the record straight. Dr McBride's work was not 'presented to the European Toxicology Society in Dublin five years ago.' According to Professor Neubert's paper, Dr McBride's data were presented in Dublin at the time of the European Teratology Society's meeting but outside the regular programme and were therefore not included in the abstracts of that meeting. The data were 'released by the authors (without knowledge of the organisers) to the press as highlight of this meeting.' There is therefore no valid experimental evidence to support the 'second generation' hypothesis.

The second strand to the media story is that a small number of people who were accepted as having been damaged by thalidomide have become parents of similarly affected children. The only reasonable conclusion is that a dominant gene is responsible in both generations. When decisions were being made in the 1960s and 1970s about who had and who had not been damaged by thalidomide, a number of difficult cases were given the benefit of the doubt (that is, attributed to thalidomide), and some decisions were made by doctors who lacked the necessary experience. It was inevitable that some of these people had not, in fact, been damaged by thalidomide.

So long as the media are prepared to perpetuate the myth of second generation defects due to thalidomide, medical scientists must spend time refuting it. The myth raises hopes of additional compensation for the few but, more importantly, it raises fears among people with defects due to thalidomide that their future children are at serious risk of being born with similar defects. Even those who have completed their families and whose children are healthy may fear third generation defects in their grandchildren.

### **Zambia: visibly crippled by debt burden**

The following extract is from the *Guardian* newspaper of Thursday 14th May, 1998:

'Non-governmental organisations such as Cafod and Oxfam say that, of all African countries, Zambia is most visibly crippled by its debt burden—now \$7.2 billion (£4.3 billion)—and by the structural adjustment targets tied to World Bank cheques.

The United Nations Children's Fund (Unicef) estimates that two-thirds of Zambians live in poverty, and there is no sign of improvement on the horizon.

The human consequences are clear. Literacy is declining because families need their children to work and cannot afford school fees. Life expectancy is down from 54.4 years in 1991 to 42.6 years in 1997. As a direct result of hospitals becoming more expensive, there are now 203 infant deaths per 1,000 births, compared to 125 in 1991. Almost uniquely in Africa, access to clean water is declining.'

### **The St Francis Leprosy Guild, United Kingdom**

The St Francis Leprosy Guild (founded 1895), 26 Inglis Road, Ealing, London W5 3RL, held its Annual General Meeting in London on May 6th, 1998. Accumulated funds were approved for leprosy centres in Africa, Bolivia, Egypt, Brazil, Indonesia, Jamaica, Madagascar, Myanmar, Pakistan, Papua New Guinea, Philippines, Thailand, Vietnam and Sri Lanka (total £283,900). The Guild aims '...to help cure people who suffer from Leprosy (Hansen's Disease), to rehabilitate into the community those who have been cured wherever possible, and when necessary to give residential support to those whose disabilities require it.' The 1997 Report to Benefactors has been issued to all members of the *International Federation of Anti-Leprosy Associations* (ILEP) and is available to others on application to the above address.

## Journals, electronic publishing and the internet

Under the heading 'Electronic, international and ready for anything', the 'Editor's choice' in the *British Medical Journal*, volume 316, 11th April 1998, ran as follows:

Today we launch the *eBMJ*, an electronic version of the journal that includes not only the full text of everything published in the paper version but also begins to use the remarkable capabilities of the internet.

Eventually, the *eBMJ* is likely to be the primary version of the journal, with the paper journal being one of its manifestations. This transformation is well under way with physics and chemistry journals, and some medical journals—for example, *Pediatrics*—are already including reports on studies (we can't call them papers any more) in their electronic version that are not included in the paper versions. Some journals are also posting reports on studies on their websites the minute they are accepted, meaning they appear electronically months before they appear on paper. This vision of an electronic future will excite some and appal others, but those who love paper and hate computers need not fear. The ease of reading and handling paper and its transportability and familiarity make it most unlikely that it will ever disappear.

But where electronic journals will go is far from clear. They might disappear altogether as authors of studies find other ways to reach readers, or we might move to a few megajournals with all the small ones disappearing. The editor of *Circulation*, the world's primary cardiology journal, thinks, for instance, that one cardiology journal is enough. To survive, journals will have to learn to use the full benefits of the web—speed, worldwide reach, infinite capacity, searchability, interactivity, the ability to link, and so on. Paper people must become web people.

One of the most obvious benefits of an electronic journal is that new material can be accessed immediately anywhere in the world. It wasn't much more than a 100 years ago that it took 18 months for a letter sent from Britain to get a reply from New Zealand. The paper version of the *BMJ* still takes well over a week to get there. The immediacy and reach of electronic publishing means that an international journal like the *BMJ* can become truly global. Already about three quarters of the roughly 20,000 people who access the *eBMJ* each week come from outside Britain, and about 40% have never seen the paper version. This issue of the journal includes information from India, France, the United States, Bangladesh, Canada, the four countries of Britain, South Africa, Norway, Poland, Thailand, Nicaragua and Cuba, Australia, and New Zealand, but perhaps an issue five years from now will be still more international. If, of course, there is one.

## Blister pack for 'ROM' treatment of single-lesion, paucibacillary leprosy

A ROM blister pack is available as a free supply from WHO, made possible by a gift from the Nippon Foundation, Japan. The single-dose combination of drugs (rifampicin, ofloxacin and minocycline) is for the treatment of single-lesion, paucibacillary leprosy only. Furthermore, WHO recommends (see *MDT: Questions and Answers*, revised 1997, *Action programme for the Elimination of Leprosy*, WHO Geneva) that this regimen may be used only by programmes detecting a large number (1000 or more) such cases annually. The relevant section on ROM from the *Seventh Report of the WHO Expert Committee on Leprosy*, *WHO Technical Report Series 874*, 1998, reads as follows:

### Regimen for single-lesion paucibacillary leprosy

There is some evidence to suggest that single-lesion leprosy is a clinical entity and may be cured by a limited amount of chemotherapy, so a separate regimen for these patients will be useful. The efficacy of a single dose of a drug combination consisting of 600 mg of rifampicin, 400 mg of ofloxacin and 100 mg

of minocycline (ROM) for the treatment of single-lesion paucibacillary leprosy has been proved in a multicentre, double-blind field trial in India. The trial involved 1483 patients with single-lesion paucibacillary leprosy, who were randomly allocated to two groups (study and control), both of which received 6 monthly doses of drug or placebo. The first group was treated with a single dose of ROM and 6 monthly doses of placebo, while the second received 6 monthly doses of WHO MDT for paucibacillary leprosy and a single dose of placebo. At follow-up, 12 months after the 6-month treatment, the treatment failure rate was identical (0.9%) in both groups; adverse effects and leprosy reactions were mild and rare in patients treated with a single dose of ROM and who did not differ significantly from those treated with the standard WHO MDT regimen. Although a single dose of ROM was marginally less effective, in terms of clinical improvement, than the standard MDT regimen, the operational advantages of single-dose treatment are enormous, especially when it is taken into account that, in some countries such as India, more than 50% of newly detected cases are classified as having single-lesion paucibacillary leprosy. The Committee considered that a single dose of ROM is an acceptable and cost-effective alternative regimen for the treatment of patients belonging to this category.

### **Seventh Report of the WHO Expert Committee on Leprosy, WHO Technical Report Series 874**

The above Report has recently been circulated and should be studied in the original by all involved in control programmes and the treatment of individual patients. We reproduce below some extracts of pages of particular interest, including a brief summary of the remarkable achievements with multiple drug therapy (MDT) to date.

#### **Introduction**

The WHO Expert Committee on Leprosy met in Geneva from 26 May to 3 June 1997. Opening the meeting on behalf of the Director-General, Dr R. H. Henderson, Assistant Director-General, noted that more progress had been made in the fight against leprosy during the past decade, since the last meeting of the Committee in 1987, than during any other period in the history of leprosy control. He also noted the reputation of the Committee in finding the right balance between scientific findings, the needs of individual patients and their communities, and the concerns for public health. He expected the Committee to address important issues relating to leprosy chemotherapy, the prevention of leprosy-related disabilities and impairments and the need to simplify approaches in order to reach all patients, including those living in remote areas.

In May 1991, the Forty-fourth World Health Assembly adopted resolution WHA44.9 declaring the commitment to promote the use of all control measures, including multidrug therapy (MDT) together with case-finding, in order to attain the global elimination of leprosy as a public health problem (reducing the prevalence to below 1 per 10,000 population) by the year 2000.

The resolution urged Member States in which leprosy is endemic:

- to further increase or maintain their political commitment and give high priority to leprosy control so that the global elimination of leprosy as a public health problem is achieved by the year 2000;
- to strengthen managerial capabilities within leprosy programmes, particularly at the intermediate level, and to improve training in leprosy for health workers at all levels, including medical students and student nurses;
- to ensure that coverage of MDT is maintained at the highest level possible and that patients comply with treatment;
- to strengthen case-finding activities through various approaches, including health education, community participation and training of health workers;

- to integrate leprosy control within general health services and provide appropriate social and economic rehabilitation measures as soon as possible in accordance with local realities;
- to improve national information systems in order to facilitate monitoring and evaluation of the elimination of leprosy;
- to coordinate the technical and financial resources made available for leprosy control by international and nongovernmental organizations so that they are utilized in the best way.

The establishment of the goal of eliminating leprosy as a public health problem has enabled countries where leprosy is endemic to increase their political commitment and priority for leprosy and to organize and intensify antileprosy activities, which in turn has resulted in a major reduction in the prevalence of the disease. The two international conferences on the elimination of leprosy organized by WHO in Hanoi, Viet Nam, in July 1994 and New Delhi, India, in October 1996 consolidated political commitment towards leprosy elimination by the countries most affected by the disease. Support for leprosy work from various participating agencies, including both national and international nongovernmental organizations, bilateral agencies and other international organizations, has also greatly contributed to the progress towards global elimination of the disease.

The purpose of this meeting of the Expert Committee on Leprosy was:

- to review the global leprosy situation and the technology available for eliminating the disease;
- to identify the remaining obstacles to reaching the goal of eliminating leprosy as a public health problem;
- to make appropriate recommendations for the future on technical and operational matters.

There have been dramatic changes in the epidemiology of leprosy following the widespread implementation of MDT, particularly in the prevalence of the disease. The problem of reaching patients living under difficult conditions and in remote areas has become a priority, and approaches are needed to address this through greater community participation and special initiatives. A WHO Study Group on Chemotherapy of Leprosy met in 1993 and made important recommendations on fixed duration MDT, which made integration of leprosy services into general health services more feasible. Since then, research on leprosy chemotherapy has increased the possibility of further simplifying approaches which would support integration. The remaining issues beyond elimination that need to be addressed include the prevention of leprosy-related disabilities and impairments, community-based rehabilitation, and sustainability of leprosy services. This calls for mobilization and improved coordination of all relevant agencies, including non-governmental organizations, to further reduce the burden of leprosy and its consequences.

## Global leprosy situation in 1997

Major changes have taken place in the global leprosy situation since the Expert Committee last met in 1987.

### ESTIMATED CASES

Estimates of the number of cases of leprosy in the world are useful for setting priorities and for planning elimination activities. The estimates presented here are based on information from national programmes and derived from registered figures, taking into account the levels of coverage by health services and of MDT.

Estimates for 1997 indicate that there are currently about 1·15 million cases of leprosy in the world, compared with 10–12 million cases in the mid-1980s. The gap between the numbers of estimated and registered cases indicates that over 260,000 leprosy cases remained undetected in 1996; of those, about 60% were living in south-east Asia.

## REGISTERED CASES

During the past 12 years, the number of registered cases in the world has fallen by about 85% in almost all countries and regions where leprosy is endemic. About 890,000 leprosy patients were registered at the beginning of 1997.

Leprosy remains a public health problem in 55 countries or areas, but 16 countries account for over 91% of the total number of registered cases, and five of them (Brazil, India, Indonesia, Myanmar and Nigeria) account for about 82%.

## DETECTION OF CASES

About 567,000 new cases of leprosy were detected in 1996. This figure has remained more or less constant over the past decade. Several factors appear to have contributed to the steady level of annual number of cases detected, including:

- increased case-finding activities as a result of intensified efforts and expanded geographical coverage, leading to identification of a high proportion of previously undetected cases;
- improved reporting systems; and
- decreased specificity of diagnosis of leprosy (by general health workers rather than specialized staff).

Intensified case-finding efforts and earlier detection of cases as a result of improved control programmes have masked the underlying global trend resulting from MDT. Because the annual detection rates are still very high in some countries or in some regions within countries, such countries may have difficulty in reaching the elimination goal and will need special attention.

Although the number of cases detected each year has remained constant, the characteristics of such cases have changed significantly. Over the past decade the proportion of new cases in children below 15 years and of patients with multibacillary leprosy have increased, while the proportion of newly detected cases with grade 2 disabilities (see section 6.2) has decreased.

## ACHIEVEMENTS WITH MDT

By the beginning of 1997, more than 8.4 million leprosy patients had been cured by MDT. Currently more than 97% of registered cases are receiving MDT. The increase in MDT coverage is a result of the efficacy and acceptability of MDT, which is fully standardized and of fixed duration. The numbers of relapses remain low, below 1 per 1000 patients per year, and drug resistance following MDT has not been reported.

However, taking into consideration that treatment is still of long duration (6 or 24 months), which is not always easy to monitor, data on MDT coverage should be interpreted with caution and, whenever possible, the coverage rate should be presented along with the completion rate of treatment by cohorts of patients starting treatment during the same year. Current data suggest that completion rates in most countries vary between 60% and 90%.

## Conclusions and recommendations

The major conclusions and recommendations of the Committee are summarized below.

1. The Global Strategy for the Elimination of Leprosy, based on the implementation of MDT with case-finding, is proving to be extremely successful in reducing the prevalence of leprosy and should be continued.
2. There is an important need to detect and treat the remaining undetected cases, for which special approaches, along with the extension of MDT services to all general health facilities, are required.



3. The progressive simplification of diagnostic and treatment techniques has continued to facilitate reaching more leprosy patients.
4. On the basis of a multicentre trial, the Committee considered that a single dose of a combination of rifampicin, ofloxacin and minocycline is an acceptable and cost-effective alternative regimen for the treatment of single-lesion paucibacillary leprosy. Furthermore, based on the available information, it is possible that the duration of the current MDT regimen for multibacillary leprosy could be shortened to 12 months.
5. There is a need for a fresh strategy for disability prevention and rehabilitation that would ensure a practical, community-oriented approach aimed at reaching the largest number of persons in need with cost-effective interventions.
6. The monitoring of elimination through essential indicators (see section 8) should continue. The information reported should be validated and analysed further by independent monitors in order to identify problem situations needing action.
7. In endemic countries, antileprosy activities should become, and should remain beyond the year 2000, an integral part of general health services and should also involve the communities to the fullest extent possible. Coordination between various agencies, including national and international non-governmental organizations, should be consolidated.
8. It is recommended that research in leprosy be continued, especially in improving patient care and in addressing issues after the goal of elimination has been reached.
9. It is important to sustain antileprosy activities beyond the year 2000 in order to deal with the remaining problems, including newly detected cases and persons with leprosy-related disabilities and impairments.

### **Forum for health information providers: steering group meeting, British Medical Association, London, UK, 30th March 1998**

A meeting of potentially far-reaching importance, chaired by Professor KGMM Alberti, President of the Royal College of Physicians of London, was held in the headquarters of the British Medical Association on 30th March, 1998. This was organized by Neil Pakenham-Walsh of 'INASP-Health', a specific programme within the *International Network for the Availability of Scientific Publications (INASP)* and Paul Chinnock, Editor of *Africa Health*. 'INASP-Health' is dedicated to the co-ordination and support of activities of health information providers in developing countries, including universal access to reliable information for health professionals.

A full account of the development and aims of 'INASP-Health' has been published in 1) *The British Medical Journal*, volume 314, 11th January 1997, 2) *WHO Liaison*, volume 8, numbers 2–3, August–November, 1997 and 3) *World Health*, 50th year, number 6, November–December 1997.

Thirty people involved in the provision of health information for developing countries were invited, including representatives of the *British Medical Journal*, *African Health*, *Medicine Digest*, *Practical Pharmacy*, Action in International Medicine (AIM), African Medical and Research Foundation (AMREF), The British Council, CAB International (CABI), Essential Drugs Project, Healthlink (previously AHRTAG), Authors Licensing and Collecting Society, Book Aid International, Department for Information Studies, Sheffield University, Footsteps/Tear Fund, International Health Exchange, London School of Hygiene and Tropical Medicine, Nigerian Medical Forum, Partnerships in Health Information, (previously SatelLife, UK), Teaching Aids at Low Cost (TALC), Liverpool School of Tropical Medicine, Neurology International Partnership Programme, South Thames Library and Information Services, Tropical Health and Education Trust, Tropical Health Technology and The Wellcome Trust.

The main objective of this first meeting was to discuss the advisability (or otherwise) of establishing a group or forum of health providers, essentially from the UK, but with the option of inviting participants from outside, to a) improve the knowledge and understanding of participants on the

needs of health information users and the most effective ways of meeting those needs, b) exchange ideas, contacts, information, avoid duplication, c) lobby international organizations and others for more resources to be devoted to health information provision and argue the case for health information needs to be given due consideration in the planning and implementation of healthcare programmes, d) facilitate partnerships between participants and/or the organisations they represent.

The initial round of 'self introductions' at the meeting revealed a vast pool of experience from many parts of the world in the origination, assembly, publication, distribution and assessment of health information/material, whilst at the same time underlining the need for health information providers in the UK to liaise more closely in order to avoid duplication and ensure that the main activities of all agencies working in this field are mutually well known.

Professor Alberti guided the discussions towards the main question to be addressed at this first meeting, namely the possible justification for the creation of a forum in the UK, with meetings (in London), on a regular basis. This was accepted and the next meeting will be held within a few months to discuss the practical steps to be taken to '... assist health information providers towards the achievement of a common goal: universal access to reliable health information'.

This may well be the first meeting of its kind held in the UK. It clearly has considerable potential for the identification of appropriate and sustainable channels for the provision of reliable health information to health workers at all levels in developing countries. *INASP-Health* is supported by the *British Medical Association* (UK), *Danida* (Denmark), and *Reuters*.

Further information: INASP-Health, 27 Park End Street, Oxford, OX1 1HU, United Kingdom. Tel/Fax +44 (0) 1865 249909/251060. Email [inasp@gn.apc.org](mailto:inasp@gn.apc.org)  
WWW: <http://www.oneworld.org/inasp/>

### **Damien Foundation India Trust: Annual Report 1997**

We are indebted to Dr P. Krishnamurthy, Secretary DFIT, India for the annual report of activities, 1997 covering projects in Uttara Pradesh, Bihar, Maharashtra, Karnataka, Andhra Pradesh, Pondicherry, Tamil Nadu and Kerala. Extracts from the Introduction read as follows:

Damien Foundation India Trust (DFIT) passed one more year of useful service to the people in different parts of the country. It went through a phase of expansion and consolidation especially in Bihar and potentiation in other areas through a shift in emphasis in operational process. New inroads were made into the leprosy problem in Bihar, new direction was given to the programme through revised strategies, new programmes were initiated, new initiative was taken to give fillip to efforts on health education, new orientation was given to Prevention of Disability (POD) programme and a new dimension was given to interaction with projects.

The prevalence of leprosy in India which was 57 per 10,000 in 1981 has come down to 5.7 per 10,000 in 1997. Total cases on record (December 1997) is 0.48 million. The spectacular fall in prevalence has been mainly due to the systematic implementation of Multi Drug Therapy (MDT) in the endemic districts. There is no state in India with a prevalence of 50 or more per 10,000. While considerable achievement has been made in some states, in others it is far from satisfactory for various reasons. The state of Tamil Nadu which was highly endemic for leprosy witnessed a dramatic fall in prevalence over 10-year period of MDT implementation and has, following intense case detection campaign, integrated leprosy control with general health services. In the meantime efforts are being made by various agencies, national and international, Government and Non-government, in some of the Northern states like Bihar to establish leprosy control programme adapting locally suitable strategies.

Damien Foundation India Trust is one of the premier voluntary agencies devoted to leprosy eradication and is contributing in its own unique way towards the common struggle against leprosy. Of the 23 leprosy eradication projects it supports, two are directly run, two are Government MDT districts and 19 are projects (three in the north) run by voluntary agencies. DFIT has also placed 15 technical

teams, each consisting of a District Leprosy Advisor and a Supervisor, in 15 districts of Bihar. All the projects that are supported are field-based.

The extent of leprosy problem in Bihar becomes apparent when one looks at the figures—22% of the case load of India and 10% of the global case load is contributed by the state. There were 122,046 cases on record with a Prevalence of 12.8 per 10,000 at the end of September 1997. It is believed that there may be twice the number of cases in the community. Of the 39 districts 17 are considered to be endemic and the rest non-endemic. Since the estimates were made years back and since the programme has not shown the expected progress it is safe to assume that differentiation of districts on the basis of endemicity becomes less relevant. In the endemic districts the programme is run vertically with staff meant only for leprosy and in the non-endemic ones it is implemented through mobile leprosy treatment units (MLTU) one for every 1,000,000 population in a district. Case detection is done through various surveys and voluntary reporting. Patients are treated at service delivery points through daily circuits.

*Further information:* Damien Foundation, India Trust, 27 Venugopal Avenue, Spur Tank Road, Chetpet, Chennai 600 031 India.

## Erratum

Uveitis in leprosy patients who got inactive condition in pre-WHO/MDT era, Namisato *et al.* Volume 69, page 82. Authors' names should read: M. Namisato, S. Joko, S. Izumi, K. Murakami and H. Ogawa.

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## CONTENTS

### 221 Editor's choice

#### Editorial

- 222 *Mycobacterium w* immunotherapy in leprosy. C. WHITTY

#### Review Article

- 225 The management of leprosy reversal reactions. W. J. BRITTON

#### Original Articles

- 235 Experimental leprosy in rhesus monkeys: transmission, susceptibility, clinical and immunological findings. B. GORMUS, K. XU, G. B. BASKIN, L. N. MARTIN, R. P. BOHM, J. L. BLANCHARD, P. A. MACK, M. S. RATTEREE, W. M. MEYERS AND G. P. WALSH
- 246 *In vitro* studies on extracellular matrix production by *M. leprae* infected murine neurofibroblasts. N. SINGH, T. J. BIRDI, S. CHANDRASHEKAR AND N. H. ANTIA
- 257 Intertester reliability of manual muscle strength testing in leprosy patients. J. W. BRANDSMA, W. H. VAN BRAKEL, A. M. ANDERSON, A. J. KORTENDIJK, K. S. GURUNG AND S. K. SUNWAR
- 267 Leprosy and disability control in the Guera Prefecture of Chad, Africa: do women have access to leprosy control services? JOHANNES SCHÄFER
- 279 Use of a whole blood assay to monitor the immune response to mycobacterial antigens in leprosy patients: a predictor for type 1 reaction onset? R. E. WEIR, C. R. BUTLIN, K. D. NEUPANE, S. S. FAILBUS AND H. M. DOCKRELL

#### Special Article

- 294 Journals on the Net: some sites reviewed. SUSAN BOOBIS

#### Further Education

- 297 Indications for surgery in leprosy. M. VIRMOND

#### Letters to the Editor

- 305 Editorials, September 1997 by W. C. S. Smith, P. Fine and D. K. Warndorff. C. R. REVANKAR

### 306 Book Reviews

#### 308 Teaching Materials and Services

LEPRA/TLM Ophthalmic Course, Karigiri, India 1998 • Research synthesis: its importance in developing countries • WHO International Exchange of Health Literature • TALC: 'Clinical Tuberculosis' by Crofton, Horne & Miller, now available in 18 different languages • Topics in International Health: 'CD-ROMs tackle global diseases': The Wellcome Trust, UK, 24 April 1998 • TALC, UK: Leprosy in childhood: colour transparency teaching set with text • ALERT Training Calendar 1999

#### 314 News and Notes

ActionAid Disability News: the Newsletter of the Disability Division of ActionAid in India • Researchers create designer antibody • WHO Committee identifies constraints that stall progress against TB • India approves leprosy vaccine • Global research funding for AIDS, malaria and tuberculosis • Scotland as a focus of world electronics industry • INASP: International Network for the Availability of Scientific Publications • Partners: Magazine for Paramedical Workers: The Leprosy Mission International • 'Dominant gene probably caused some of the defects ascribed to thalidomide' • Zambia visibly crippled by debt burden • The St Francis Leprosy Guild, United Kingdom • Journals, electronic publishing and the internet • Blister pack for 'ROM' treatment of single-lesion, paucibacillary leprosy • Regimen for single-lesion paucibacillary leprosy • Seventh Report of the WHO Expert Committee on Leprosy, WHO Technical Report Series 874 • Forum for health information providers: steering group meeting, British Medical Association, London, UK, 30<sup>th</sup> March 1998 • Damien Foundation India Trust: Annual Report 1997 • Erratum • Recent publications