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Editorial

APPLICATION OF MONOCLONAL ANTIBODIES TOWARDS IMMUNOLOGICAL STUDIES IN LEPROSY

Immunology has a key role in the study of leprosy. Unlike any other infectious disease, the immune system decides not only whether the outcome from contact with the infection will lead to protection (self-healing) or disease, but also dictates the pattern of clinical manifestations. The clinical classification of forms of leprosy has been based fundamentally on shifting balance between T-cell-mediated and humoral reactions to *Mycobacterium leprae*.[1] This classification has been widely accepted apparently as the best guidance to the two polar or borderline types of the disease. However, it is surprising that these investigations often refer to the response of the immune system to *M. leprae in toto*, with only scant attention to the fact that diverse reactions may arise towards the antigenic determinants (i.e. epitopes) of the various protein, glycolipid and polysaccharide constituents[2] of the leprosy bacillus. Since lymphocytes react towards these epitopes of the bacillus individually, it seems conceivable that those immune reactions which play a critical role for the pathogenesis of leprosy would not be random but rather restricted in specificity to certain structures of the leprosy bacillus.

Progress in the analysis of the specificity of cell-mediated and humoral immunity in leprosy has been slow and further advance could be expected only from the definition and purification of the relevant antigens. The purpose of the subsequent discussion will be to bring attention to a novel technology, i.e. hybridoma cell line derived monoclonal antibodies (MAB), which could greatly contribute towards the identification of those antigens which are functionally important for the development and progression of the respective forms of the disease.

General principles and technology

Antisera from immunized individuals represent 'polyclonal antibodies', as they

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are derived from multiple clones of B cells, each responding to the individual epitopes of *Mycobacterium leprae* and even heterogeneous in response to one epitope in terms of affinity and isotype. Monoclonal antibodies are uniform in specificity and structure, being the product of a single B-lymphocyte clone which has been 'immortalized' by fusion with an autonomously growing myeloma cell. The technology has been well described in various excellent reviews and monographs.[3,4] Briefly, spleen lymphocytes harvested from immunized mice are fused in the presence of polyethyleneglycol with myeloma cells which are deficient in the enzyme hypoxanthine guanine phosphoribosyl transferase (HPRT). Deficiency in this enzyme of the 'salvage pathway' of nucleic acid synthesis will selectively prevent myeloma cells from growing in selective culture media (HAT) containing aminopterin which blocks the main biosynthetic pathway of nucleic acids whilst allowing the growth of hybrid cells which had acquired HPRT from the lymphocyte fusion partner.

The actual fusion manipulation takes less than 1 h to perform. The cells distributed at 10^5–10^6/per well frequency in plastic trays will manifest macroscopically discernible colony growth within 7–14 days. At this stage, culture media are tested for the presence of antibodies by a sensitive test, e.g. radioimmunoassay and the cells from antibody-positive wells are cloned by limiting cell dilution. Stable viable cell lines, usually of lower yield than the number of positive primary wells, are stored frozen in liquid nitrogen and grown as ascitic tumour in mice which generates ascites fluid for bulk production of the monoclonal antibody.

Review of reported MABs to *Mycobacterium leprae*

The salient characteristics of MABs described in recent publications[5–7] are summarized in Table 1. Although only one representative MAB clone for each specificity is listed here, several other hybridomas of apparently overlapping specificity were generated at least for the *M. leprae*-specific MY1a and MY2a specificities.[6] The specificity of MABs was designated by prefix MY (mycobacterial) and an arbitrary number, defining the distinct molecules (e.g. MY1, MY2, etc.); the letters distinguish between the epitopes expressed on the same molecule (e.g. MY4a, MY4b). It would be beneficial upon agreement with other investigators to compare reagents and to introduce a common nomenclature. The 68Kd molecule carries at least one identified species-specific (IVD8) and one cross-reactive (IIH9) epitope.[5] Similarly, it is likely that MY1 (12Kd) and MY2 antigens would carry in addition to their species specific epitopes other determinants possibly of the cross-reactive type. It is of interest, that all anti M1a and M2a hybridoma lines were generated from spleens of mice which had been immunized with the supernatant fraction of sonicated *M. leprae*, whilst spleens from whole sonicate injected mice yielded only cross-reactive MABs.[6] Conceiv-

Table 1. Review of monoclonal antibodies to *Mycobacterium leprae*

Monoclonal antibody code/specificity-class	Chemical structure–molecular weight	Localization	Cross-reactivity	Ref.
ML06/MY1a-IgG1	Protein-12Kd	Cytoplasm	None	6
ML04/MY2a-IgG1	Protein	Cytoplasm(?)	Marginal§	6
ML30/MY3a-IgG1	Protein-35-70Kd†	Cell wall	Broad	6
ML02/MY4a-IgG3*	Polysaccharide-40-50Kd‡	Cell wall	Broad	6
ML34/MY4b-IgM*			Broad	6
IVD8-IgG1	Protein-68Kd	Cytoplasm	None	5††
IIH9-IgG1			Broad	5
PG_2B8F-IgM	Glycolipid	Cell wall	Marginal**	5††
AM-8-2C2-IgM	Arabinomannan	Cell wall	All mycobacteria	5††
A-494-IgM	?	Cell wall	All mycobacteria	7

* Precipitating antibody.
† Multiple bands.
‡ One broad band.
§ *M. kansasii*, *M. avium*, *M. paratuberculosis*.
** *M. bovis*, *M. nonchromogenicum*, *M. terrae*.
†† Buchanan TM, Young DB, Miller RA and Khanolkar SR. Personal communication.

ably, the presence of cell-wall constituents could have suppressed the murine antibody response to the species-specific protein antigens.

TAXONOMIC ASPECTS

It may seem disappointing that none of the MABs can 'type' for an antigen which would distinguish between slow and fast growing species of mycobacteria. Neither is there a linkage with any of the defined biochemical markers. The two *M. leprae*-specific epitopes, MY1a and MY2a are not expressed by mycobacteria strains ICRF or W which have been implicated for closer relationship with *M. leprae*, but their presence in 'leprosy-derived corynebacteria'[8] is yet to be determined.

It is of interest to characterize the antigens with epitopes which are shared between *M. leprae* and the cultivable species of mycobacteria. Antibodies of restricted cross-reactivity are of best use here.[6] The MY2a epitope is marginally represented only on 3 other species, namely *M. kansasii*, *M. avium* and *M. paratuberculosis*. The MY3a epitope varies quantitatively between several species and may be of interest for experimental models since it is expressed strongly on *M. lepraemurium*. The best 'typing' pattern is demonstrable with MABs directed towards the two distinct epitopes of the MY4 polysaccharide antigen which segregate in several species of mycobacteria almost in a reciprocal manner. The

MY4a epitope is most pronounced on mycobacterium strain W and also on *M. avium* and *M. kansasii* whereas MY4b has the strongest expression on *M. tuberculosis*, *M. bovis*, *M. scrofulaceum* and *M. paratuberculosis*. The relevance of these relationships and their possible utility in immunization programmes is yet to be explored. Hence, it may be worthwhile to determine the immune response to a shared epitope of *M. leprae* when presented in the context of another mycobacterial species.

Antigen deposits in tissues

Probably the most ominous outcome of anergy in lepromatous patients relates to the impaired clearance of *Mycobacterium leprae* bacilli. Although the histological localization and amount of whole or fragmented bacilli have been examined previously, little is known about the composition of the retained antigens in tissues. Nevertheless, this may be important, since glycolipids have been implicated in the induction of granulomas. Immunohistological studies using enzyme-labelled MABs binding to distinct antigens are now technically attainable. This approach should be superior to acid-fast staining which may no longer detect certain constituents or to staining with polyclonal antisera which do not discriminate between the multitude of antigens. It would be of particular interest to analyse the antigenic composition in patients with tuberculoid or indeterminate leprosy where the deposits are infrequent and small. However, the paucity of bacilli does not exclude the possible role of a particular mycobacterial antigen *in situ*. The role of deposited antigen or immune complexes has been implicated for the pathogenesis of erythema nodosum leprosum. If the clinical manifestations result from 'slow degradation of large amounts of antigen'[9] it would be pertinent to identify the constituents which are most resilient to breakdown and possibly with biological (e.g. adjuvant) activity. This is relevant also to chemotherapy which may gradually eliminate viable bacilli but leave certain cell-wall constituents to persist for prolonged periods.

Circulating immune complexes

Their composition in sera of patients with lepromatous leprosy has been analysed recently.[10] The results indicated that the number of mycobacterial constituents in these complexes is rather restricted and so far only one protein antigen with a molecular weight of about 67Kd has been identified. Monoclonal antibodies directed towards two distinct epitopes of an antigen of corresponding molecular weight have been described[5] and if their binding to complexed antigen is established, they could be valuable specific tools for monitoring the levels of circulating complexes.

Serological assays for leprosy

Antibody levels towards antigens of *Mycobacterium leprae* have been studied extensively by several previous investigators who found that they become elevated consistently in patients at the lepromatous end of the clinical spectrum. It was considered that antibody levels may help to monitor the bacterial load or may predict the possibility of a relapse during treatment. However, the employed serological tests were more or less lacking in specificity. A novel serological test based on the use of species-specific monoclonal antibodies has been developed recently.[11] In this assay, the binding of a radio- or enzyme-labelled MAB (ML04) to wells of microtiter plates which had been precoated with *M. leprae* soluble antigen is competitively inhibited by serially diluted sera from patients. Thus, only those human antibodies which have specificity matching the combining site of the labelled MAB probe would give positive inhibitory values. Protein molecules from *M. leprae* may carry both species-specific and cross-reactive epitopes which in turn would break down the specificity of a direct antibody binding test even if the antigen was purified to homogeneity. This problem does not affect the serum competition test in which the epitope specificity is safeguarded by a selected specific MAB. Using this test, almost all tested LL patients showed ML04 binding inhibitory antibody levels.[11] Preliminary results have shown demonstrable antibody levels also in a certain proportion of tuberculoid patients and even in healthy family contacts of leprosy patients (Sinha and Sengupta, in preparation). These pilot studies are encouraging and require further evaluation in long-term prospective clinical trials. The merit of the test could be prognostic, whereby an increase in antibody levels would indicate the shifting of the disease towards the lepromatous pole of the spectrum. However, epidemiologically the most valuable outcome would be if the test, on the strength of its specificity, would differentiate (a) between infected and non-infected healthy subjects; or (b) predict on a prognosis for self-healing or progressive disease in endemic areas.

Potentials for a *Mycobacterium leprae* specific skin test and lymphocyte stimulation test (LST)

The 24–72 h skin erythema and induration reaction to intracutaneous injection of the soluble fraction of sonicated *M. leprae* (i.e. Dharmendra's antigen, or leprosin) represents a T-cell mediated, delayed type hypersensitivity reaction which reflects the response to previous *M. leprae* infection. Earlier attempts with the fractionation of these crude bacterial extracts met with only partial success.

For epidemiological and diagnostic purposes, it is clearly desirable to distinguish between previously *M. leprae* infected and non-infected, yet clinically healthy persons in both endemic areas. This seems feasible in the light of the

report that non-exposed individuals with high LST responses to PPD showed an almost complete lack of 72-h skin reactions to the soluble fraction of sonicated *M. leprae* hence suggesting the existence of T-cell stimulating, *M. leprae* specific antigens.[12] However, others reported that lymphocytes from the majority of non-exposed healthy subjects showed proliferative responses to soluble sonicated *M. leprae* antigen.[13,14] Therefore, the diagnostic reagent to sustain an *M. leprae* specific test needs further purifications and structural studies. Since the T-cell responses to mycobacteria are mediated by protein molecules and not by the polysaccharide or glycolipid constituents, the MAB-defined MY1 or MY2 molecules when isolated and supplied in adequate quantities, appear to be possible candidates for a specific skin test. So far, we attempted in our laboratory the purification of MY1 by MAB-based affinity chromatography and achieved about 10% yields of partially purified antigen. This material when tested in guinea-pigs showed cross-reactivity with PPD, but it was not possible to decide whether that was attributable to cross-reactivity of the MY1 molecule or of the contaminating antigens (R J W Rees, unpublished results).

Studies with other protein antigens have suggested that the epitopes recognized by B and T cells respectively are structurally distinct. Although the molecules which carry the *M. leprae* specific epitopes recognized by MABs remain the best candidates for the search for T-cell stimulatory structures, it may be necessary to carry out this analysis further at the level of peptide structure.[15,16]

Analysis of T-cell anergy in lepromatous leprosy

A controversy in this area relates to the degree of specificity. Anergy may be (i) clonally restricted to either *Mycobacterium leprae* specific[17–21] or to common mycobacterial antigens,[22] or (ii) a polyclonal defect in the T-cell immune system. The cellular mechanisms are considered to be either (a) deletion of the responding helper (T_H) cells,[19,21] or (b) activation of suppressor (T_S) cells.[17,23]

It has been demonstrated in experimental models that T_S cells can be specific to one molecule of a cellular antigen or even specific to a particular epitope of a protein antigen.[24] It would be feasible to determine whether any of the MAB-defined epitopes activate T_S cells. Here, the primary interest in the *M. leprae* specific epitopes is supported by the results obtained by LST as well as by skin reactions that anergy is induced by *M. leprae* soluble antigen but not by PPD.[18,19,21] Thus, *M. leprae* specific epitopes may be activating T_S cells which effect directly[17] or via macrophages[23] the suppression of the mitogenic response to antigens represented in other species of mycobacteria and also the polyclonal response to Concanavalin A. It is important to distinguish between the highly specific activation stage and the much less restricted effector stage of suppression. Interestingly, the macrophage lysate mediated suppression is not entirely

non-specific,[23] since suppression was most pronounced in response to *M. kansasii* and *M. avium*, both of which express, at least weakly, the MAB defined MY2a epitope.

Long-term dapsone therapy causes a reversion of T-cell anergy to common mycobacterial antigens but leaves a persistent suppression of the *M. leprae* specific response.[25] This change in the specificity of anergy may be monitored as an appropriate parameter, possibly of prognostic value.

Prophylactic immunization versus immunotherapy

Acquired T-cell mediated immunity to mycobacterial infections is thought to confer protection against infections through activation of macrophages. However, it is understood that 'hypersensitivity' reactions as measured by the LST do not correlate with 'protective' immunity, since LST values are raised in many TT/BT cases with destructive disease whilst the values are negative or low in indeterminate patients, most of whom are self-healing. The failure of delayed type hypersensitivity to suppress mycobacterial growth was shown also in murine experiments.[26] It has been speculated in leprosy (as in tuberculosis) that hypersensitivity and protective immunity may be directed towards separate antigens of mycobacteria.[27] These thoughts were not ascertained experimentally presumably because of the lack of adequately purified antigens; nevertheless, the subject yet remains pertinent for study with MAB-defined antigens.

It may appear that prophylactic vaccination and immunotherapy, i.e. conversion of anergic lepromatous patients, represent different categories. However, a sharp differentiation may not be necessary when considering that potential anergy based on environmental or genetic grounds could represent the main problem also in the susceptible healthy individuals from endemic areas (a minority of the total population). Indeed, only a single rationale, embraced by the inoculation of live BCG with or without killed *Mycobacterium leprae* has so far been explored with partial success in prophylactic[28] and 'therapeutic' immunizations[29] as well as in murine experimental work.[30] Elucidation of the mechanisms of immunotherapy which is of key importance, may benefit from the use of molecularly defined antigens. Unlike other conventional vaccines, the optimal strategy for the pre-emption or reversion of active suppressor cells is yet to be defined.

Synopsis

Monoclonal antibodies produced by hybridoma cell lines are of restricted and uniform specificity. They represent reagents which are in many aspects superior to the heterogeneous mixture of 'polyclonal antibodies' present in antisera from immunized or infected individuals. The technology for producing MABs is now

firmly established, relatively easy to perform and require facilities for tissue culture, immunoassays and a supply of an inbred strain of mice or rats. Three protein antigens (MY1-12K, MY2 and 68K) carrying distinct antigenic determinants, which are expressed by *Mycobacterium leprae*, but not by several other species of mycobacteria, have been identified by MABs. These *M. leprae*-specific determinants may help to make important advances in: (1) detection of antigen or immune complexes in tissues or body fluids; (2) serological diagnosis, disease monitoring and epidemiology; (3) development of a specific skin test; and (4) further research on therapeutic or prophylactic immunization against leprosy. So far, a serological test based on the use of the ML04 (anti-MY2a) monoclonal antibody has been developed and evaluated in a pilot study.[11] The diagnostic potentials of this test as well as the various other possible applications of MABs deserve attention in future studies.

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Blindness in leprosy: a report on evaluation and physical rehabilitation methods

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Summary A report is presented on rehabilitation measures used in 18 blind leprosy patients with primary and secondary deformities. The nature of their physical dysfunction, assessment methods used, effect on activities of daily living, treatment techniques to improve independent function and their effectiveness and acceptability by patients are all discussed.

The whole study is in the social context of South India and the geographical environment of a rural area.

Introduction

Of all the senses vision is the prime receiver.[1] It is the synthesizing sense enabling priorities to be established and acted on. Leprosy produces a crippling disability by inducing blindness and loss of sensation in the extremities. Statistics suggest that 5% of persons with leprosy will ultimately become blind.[2] By that time most of them are aged and totally crippled from primary and secondary deformities due to loss of sensation, muscle wasting, lack of joint movement, absorption, reaction and ulceration in the extremities. Also, such patients lose confidence in their remaining senses, contact with the environment, written communication and the basic skills which they knew beforehand.

The following report is based on our 22 months' experience in rehabilitating 18 blind leprosy patients. The objective of this is to make detailed assessments to find out the existing ability and talents of the patients and to make use of these to the maximum for retraining independence in personal care and mobility.

The methods we have used are designed for those who will return to their own homes after rehabilitation, so some of the methods will not be applicable to patients living in institutions where the inmates help each other under conditions where functional independence is not such an important factor.

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Assessment and training

SUPERFICIAL AND DEEP SENSITIVITY TESTS AND TRAINING

The non-leprosy blind person compensates for the loss of vision by developing 'fine finger-tip-touch sensibility'. Most of the blind leprosy patients we dealt with lack cutaneous sense in their fingers. If they possessed sensation, functional independence could be gained easily.

Strengthening tactile sense is done by carefully judging the sensation available and improving its perceptional ability. The method used is to ask the patient to match simple pattern puzzles using the area of islands of sensation which he has. This can be up-graded to more complicated pattern puzzles. Also, handling varieties of texture boards and blocks of various sizes and shapes is useful.

Trailing the patients hand along all the beds and lockers of his ward, along the walls of the ward and toilet interior, and on objects such as switches, windows and taps, will give a mental picture of the whole ward. This encourages the patient to learn to use his tactile and proprioceptive sense.

FUNCTIONAL AND SELF-CARE ASSESSMENT AND RE-TRAINING

The way the patient manages various self-care activities will give some information as to his complete or partial dependency, slowness in performance and/or dangers in the manner of performance, etc. Self-care assessment charts should be used to avoid missing any details. Feeding, dressing, toilet management, ambulation and travelling are the basic functional activities to be assessed in details. A four-point grading such as 1, normal; 2, moderately slow; 3, very slow; and 4, unable, is found easy to use.

The aim of this self-help training is to make the patient independent in those activities in which he has previously been totally dependent on other people, and to modify and improve the activities which the patient is performing in a clumsy, slow or dangerous way. Always suggest more than one way of doing things so that the patient can select.

Feeding

Teaching the patient to eat with the spoon instead of his fingers is advisable. The patient should be taught to keep the plate, mug, spoon, etc., in a certain order so that he knows where they are. Localizing these objects is done by gently tapping around the area where they are kept (Figure 1). To pick up the spoon he has to grossly grasp in the direction of the sound. To confirm whether he has grasped it, sensation of the lips is used (Figure 2). The patient holds it properly by holding the mouthpiece of the spoon in between his teeth and then he grasps it firmly in the dominant hand. Allow the patient to use the type of grasp he is accustomed to.

Figure 1. Identifying the position of the spoon by auditory clues.

If the patient is unable to pinch due to shortening of thumb and fingers a 'U'-shaped spoon handle with padding will be found useful (Figure 3).

A mug after being located, as above, is grasped firmly with the right hand, inserting the fingers of the left hand into its handle. Before lifting it the patient has to make sure that he is holding it firmly enough by slowly lifting it a little and feeling its weight by the proprioceptive sense.

Dressing

Shirts and blouses should be a little larger than the actual size of the patient. In these, buttons and button holes are replaced with 'Velcro'. To put on these

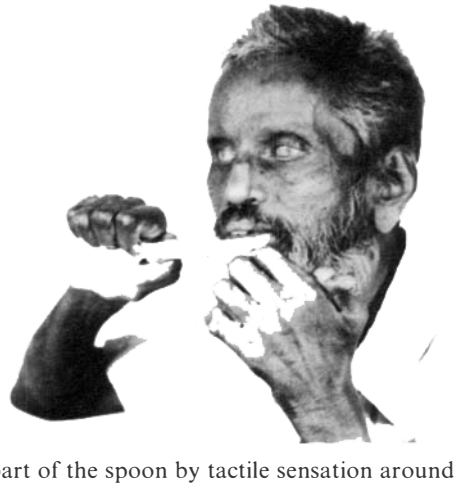

Figure 2. Identifying the part of the spoon by tactile sensation around the lips.

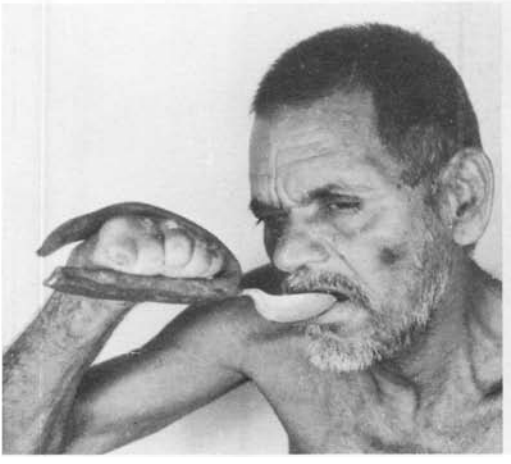

Figure 3. 'U'-shaped spoon.

Figure 4. Identifying the centre of the collar.

garments the patient feels the centre of the collar with his lips and clenches it in between his teeth, then puts his hands into the sleeves (Figure 4). Fastening is done by grasping the edges of the shirt and with the lips identifying the texture of the 'Velcro' attached on each side. Pulling the ends of the garment apart will unfasten it and the patient can find his own way of removing it.

Toilet

In their home environment most patients defaecate in the open fields in a squatting posture. The common problem all of them expressed was difficulty in walking on the ploughed, uneven ground to reach the habitual site to defaecate. This was solved by teaching them to use a cane as a guide over uneven ground. If they still have difficulty, an additional walking stick is given to stabilize them, holding the stick in the non-dominant hand, and the cane in the dominant hand. Some patients with squatting difficulties have been given a locally produced, lightweight commode which may be kept behind a bush and carried to the habitual site when its use is required.

Written communication

For literate patients, their abilities in written communication before becoming blind should be noted and maintained. We never tried braille or touch typing because of the anaesthesia of the fingers, and also because of the late onset of the blindness but we have had some success in the use of cut-out X-ray films (Figure 5(a) and (b)).

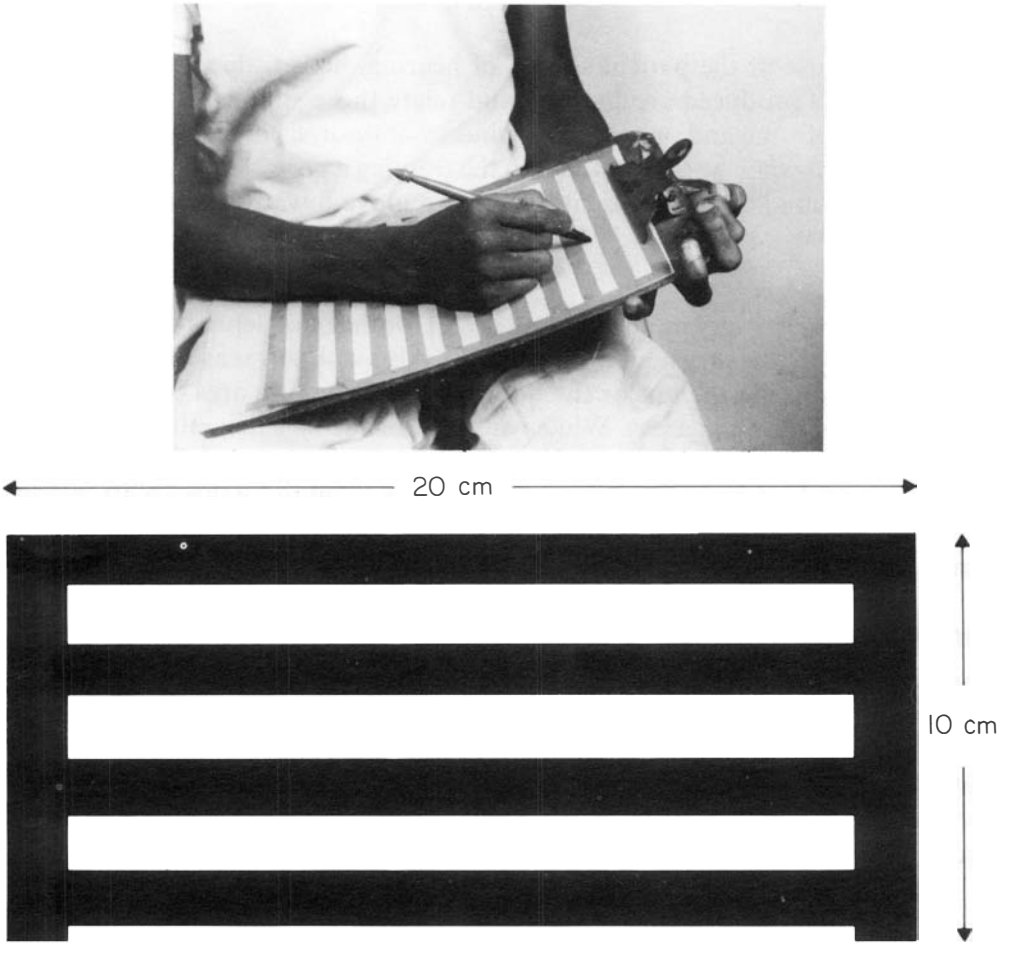

Figure 5(a). Used (discarded) X-ray film is used to aid writing for the blind patient. Using a sharp knife and a pattern, apertures are cut in the film so that it can then be placed on a sheet of paper and held in position by a clip or drawing pins. **(b).** Photostat copy of a piece of X-ray film with three apertures; a convenient size for the original is about 20 × 10 cm. The majority of patients have some degree of residual sensation in one or more fingers, enabling them to locate the edges of the film. Even with profound anaesthesia, however, the impact of the pencil against the edge of the film gives many patients (perhaps at muscle spindle level) some idea of their position on the paper.

COMPENSATION THROUGH THE REMAINING SENSES

The object of this training is to improve the ability of the remaining senses such as hearing, smell and tactile sense around the lips and the islands of sensation in the palmar surface of the hand if present, as a compensation for the loss of vision and other disabilities. Training for each of these senses is as follows:

Hearing

To make full use of the patient's sense of hearing, he is trained to identify the various sounds produced around him and relate those sounds to their sources. For example, closing and opening of a window or door, listening to the sound an object produces when he taps it. Later in the training a person stands at a distance producing a sound and the patient is asked to move towards the sound.

Smell

The sense of smell is very important and useful for the blind leprosy patient and it enables him to detect very hot objects and fire. Also with this sense he can identify different areas inside the hut or the house (i.e. the cooking area has a different odour from the sleeping area). While walking outside he can confirm or select a particular area by identifying the smell which comes from it, like a public lavatory, fruit shop, provision shop, etc. Training to improve this ability is done by taking the patient in a wheelchair to different parts of the hospital, like the garage and the kitchen, and asking him to identify the area he is in.

Light perception

These patients often have some amount of ability to perceive light, as in leprosy only the cornea is affected and not the retina. Assessment of this is made by exposing patients to various lights, such as daylight passing through doors and windows, and light from a 40-Watt bulb, to find out whether the patient can perceive these. Sense of direction can be taught efficiently with this perceptional ability.

Mobility and orientation of the environment

This means making the patient independent enough to move around freely and safely.

Initially, to make the patient mobile inside the ward, sighted guide techniques should be used. This technique involves asking the patient to grip above the left elbow of the guide and walk one step behind the guide. When he is taken for a walk the guide should narrate the destination and the objects at either side and also ask the patient what he has perceived using his remaining senses, such as light, olfactory and auditory perception. Sighted guide mobility training will reduce the fear of injury while walking.

TYPE OF CANE

To any blind person a cane is the instrument by which he can be really mobile. Bamboo canes are widely used in India by blind persons, but for blind leprosy

persons aluminium canes with nylon or metal caps* are found slightly more advantageous than the bamboo cane for the following reasons: 1 Tapping with an aluminium cane can give a better auditory clue than a bamboo cane. 2 Vibrations of an aluminium cane are far superior to that of a bamboo cane. The vibrations conducted by an aluminium cane while tapping will also give additional clues as to the type of ground on which the blind person is standing. 3 Patients with a weak grip will find it easier to hold and handle aluminium canes, which are lighter in weight.

CANE GUIDE TECHNIQUE

The majority of our patients come in with bamboo canes which are used more as a support rather than as a guide. When the bamboo cane is replaced by an aluminium one the patients prefer the latter for its lightness and comfort in handling.

There is very little variation from the standard cane guide technique. The height of the cane should be up to the patient's sternum level. Indoor and smooth surface mobility is obtained by gently sliding the nylon cap of the cane on the ground, 2 feet in front and in between the legs, to make sure of the ground and of any obstruction in his path. Outside and on uneven surfaces mobility is achieved by tapping the approximate area where the leg has to be placed. This is to make sure, by using vibration and auditory sense, that the leg can be placed safely on that area.

Learning cane guide mobility takes approximately 4–6 weeks of training. This is started with teaching the technique of smooth surface walking and progresses to uneven ground walking.

HOME VISIT

We found that at least one home visit is essential to solve a number of functional problems a patient is likely to face in his home environment. It will teach the inmates of the home, who commonly live in a joint family manner, how to assist their severely handicapped relative. In our experience, the optimum time for this visit is within 2–6 months after hospital discharge. If it is delayed beyond this period the patients, in most cases, tend to forget some of the skills learnt, the reason being that they had problems adapting such skills to suit their domestic life.

These visits showed that dressing, eating and mobility inside their homes were the activities in which most of them were totally independent or had very minimal problems, but many expressed inability or great difficulty in bathing and toilet.

* In India an aluminium cane costs approximately £2.

PREVENTION OF INJURIES

The occurrence of injuries is a great problem with this group of patients. The usual practice of teaching prevention of injury by compensating for loss of sensation with sight is not possible with blind leprosy patients. We tried some of the following methods with very limited success and the study is still in progress.

The methods are: (a) Identifying the islands of sensation present to the patients and training them to perceive through them. (b) Perceptional training to use the sensation present at the dorsum of hands. (c) Using a sensory re-orientation technique.[3] This technique could be of some use in teaching patients to perceive using the deep senses like joint, muscle stretch and deep pressure sense with which they can compensate for the loss of cutaneous sense.

Conclusion

Initial functional independence was achieved by training patients in mobility on even and uneven ground and orientating them to the hospital environment. Re-education in feeding, dressing and toilet activities were sufficient to lessen the burden on the family members of looking after a severely crippled relative.

The average time taken for training is about 100 hours, at the rate of two sessions per day, extended for 8–10 weeks. It was found that if there is a break in it for more than a month, for reasons like strict bed rest for ulcers or acute reactions, patients tend to forget most of the skills they have learnt before, and the training has to be started from the beginning again. If their stay is continued even after rehabilitation measures for other medical reasons, the training should be continued until their hospital discharge.

Acknowledgment

I thank Dr E P Fritschi, FRCS, for his assistance, guidance and continuous encouragement.

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Effects of *Mycobacterium leprae* antigens on the *in vitro* responsiveness of mononuclear cells from armadillos to Concanavalin-A

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Summary Armadillos, immunologically intact animals, develop a disseminated disease analogous to lepromatous leprosy in man when experimentally infected with *Mycobacterium leprae* of human origin. However, some animals show resistance to such infection. In this experiment the suppressive effect of *M. leprae* on Con-A-induced *in vitro* proliferation of mononuclear cells, drawn from both susceptible and resistant armadillos, is investigated.

Peripheral blood mononuclear cells from armadillos which were resistant to infection with *M. leprae* consistently showed suppressed responses to Con-A when concomitantly exposed to antigens of *M. leprae*. Armadillos with disseminated *M. leprae* infections had mononuclear cells which responded to *M. leprae* by suppressing responses to intermediate doses of Con-A, enhancing the response to an optimal dose of Con-A, and inducing no significant change in response to a minimal dose of Con-A.

These observations in armadillos support the reports in human leprosy studies that *M. leprae*-induced suppression of a Con-A response is associated with resistance.

Human leprosy is a spectral disease where paucibacillary tuberculoid leprosy, and multibacillary lepromatous leprosy are associated with high and low resistance respectively. Polar lepromatous leprosy (LL) is characterized by the nondetectability of cell-mediated immunity to the antigens of *Mycobacterium leprae*. Both *in vivo* and *in vitro* studies strongly implicate a T-cell associated defect in LL patients; however, the precise nature of this antigen specific T-cell deficiency is unknown. In recent years several laboratories have investigated the role of suppressor T-cells in leprosy patients. Using various *in vitro* functional studies, reports have shown that *M. leprae*-induced suppression of a mitogen or antigen response is associated with patients with potential resistance (tuberculoid)[18, 23] in individuals with presumably effective immunity (lepromin positive normal individuals),[23] and in occupationally exposed and presumably subclinically

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infected healthy subjects.[24] *M. leprae*-induced suppression of a Concanavalin-A (Con-A) mitogen response was conspicuously absent in disseminated multibacillary lepromatous leprosy.[18,23] It was further shown using co-cultures of lymphocytes from HLA-D matched siblings that hyperactive clones of suppressor cells were not present in lepromatous patients.[19,25] On the other hand, results from a series of elegant studies indicate that *M. leprae*-induced suppression of a Con-A response is associated with TH_2^+ cells.[16] TH_2^+ cells which suppress in the presence lepromin were demonstrated in most American and Venezuelan patients with lepromatous leprosy, but were absent in tuberculoid patients or normal controls.[15]

Clearly there are distinct differences in experimental results using co-culture techniques with Con-A and *M. leprae* antigens to test for suppressor cell phenomena in human leprosy. In view of these conflicting data, we considered it relevant to use an animal model and avoid some of the inherent variabilities in the investigation of human disease.

Armadillos (*Dasypus novemcinctus*, Linn.) are immunologically intact animals which (a) develop a disseminated infection with *M. leprae*,[11] (b) display marked differences in susceptibility to leprosy,[12] (c) have histopathological evidence suggestive of indeterminate leprosy in man[13] and responses showing both reversal and erythema nodosum leprosum reactions.[9] Although not bred under controlled conditions, armadillos from a given locale and housed after captivity in a uniform environment might be expected to be relatively uniform in their exposure to environmental mycobacteria. For experimental purposes, they have the advantages over humans with the disease in that they have a known, quantitative exposure to *M. leprae* and they can be studied in the absence of drugs. These animals, therefore, seem to offer a suitable model in which to test the hypothesis that suppressor cells are associated with susceptibility to disseminated leprosy.

Using the *in vitro* system advocated by Mehra *et al.*[15,16] to demonstrate *M. leprae* specific suppressor cells in peripheral blood, we have studied armadillos with disseminated leprosy and compared them with armadillos from the same colony which had demonstrated resistance to the disease. In this system, ^{3}H-thymidine incorporation is measured in replicate sets of mononuclear cell cultures, one exposed to Con-A alone and one exposed to Con-A plus sonicated Dharmendra lepromin. A reduction in ^{3}H-thymidine incorporation in the cultures containing sonicated Dharmendra lepromin is interpreted as meaning that *M. leprae* has specifically triggered suppressor cells with non-specific suppressor effects.

Sonicated Dharmendra lepromin consistently suppressed the *in vitro* mononuclear cell responses to Con-A in armadillos which have shown resistance to infection with leprosy bacilli. In armadillos with disseminated *M. leprae* infections, sonicated Dharmendra lepromin had mixed effects on the response of mononuclear cells to Con-A. These effects varied with the concentration of Con-A used *in vitro*.

The present investigation in armadillos supports reports that *M. leprae* induced suppression of mitogen responses is associated with the development of resistance.

Materials and methods

ARMADILLOS

Armadillos were purchased from local trappers and were all captured within a 30-mile radius of Carville Louisiana. They had been housed at Carville for at least 1 year at the time they were studied. Husbandry procedures have been described.[26]

Eighteen armadillos were designated as susceptible to leprosy and were defined as those having a systemic infection with *M. leprae*. At the time of study, these animals had received IV injections of 10^7 to 10^8 viable *M. leprae* 9–12 months earlier. They harboured $3{\cdot}06\ (\pm 2{\cdot}09) \times 10^9$ (mean $\pm$ SD) *M. leprae* per g liver, $1{\cdot}13\ (\pm 7{\cdot}02) \times 10^9$ per g spleen and $3{\cdot}26\ (\pm 2{\cdot}21) \times 10^{10}$ per g of lymph node tissue. The bacilli from these animals oxidized D-dopa,[22] and their acid-fastness was lost after pyridine treatment.[17] Each of the susceptible animals was tested once at the time of sacrifice for harvesting *M. leprae*.

Five armadillos were designated as resistant to leprosy, and were defined as healthy, long-term survivors of challenge inoculations with viable *M. leprae*. Three (Nos 199, 321 and 323) had been vaccinated with 3×10^8 heat-killed *M. leprae* in incomplete Freund's adjuvant. Armadillo No. 199 had been challenged with 10^6 viable bacilli and Nos 321 and 323 challenged with 6×10^3 viable bacilli. At the time of study, armadillo No. 199 was apparently healthy 7 years after challenge and Nos 321 and 323 were apparently healthy 3 years after challenge. The other two resistant animals were not vaccinated. One of these, armadillo No. 40, has survived in apparent good health for 10 years after being inoculated intradermally with 2×10^8 viable *M. leprae*. Four other animals receiving the same inoculum had succumbed to systemic infection with *M. leprae* 1–3 years after inoculation. The other, No. 301, was one of five apparently healthy animals 5 years after intradermal inoculation of a group of 19 with 10^4 viable *M. leprae*. The remaining 14 animals in this group developed disseminated leprosy 1–5 years after inoculation. Armadillo No. 199 was tested three times, No. 321 twice, No. 323 four times, No. 40 twice and No. 301 twice.

Mitogens and antigen(s)

1 Concanavalin-A (Con-A)—(highly purified Type IV, Sigma Chemical Co., St Louis, MO, Lot No. 58C-7700).
2 Dharmendra Antigen(s).

Acid-fast bacilli were harvested from 12 g of infected armadillo spleen tissue containing 2×10^{10} AFB/g (Lot A) and 12 g of infected armadillo lymph node containing 1×10^{11} AFB/g of tissue (Lot B). These tissues were negative for bacterial growth on fluid thioglycollate (DIFCO Laboratories, Detroit, Michigan), Trypticase™ Soy Broth and Mycobactosel™ L-J Medium Slants (BBL, Cockeysville, MD). The non-cultivatable acid-fast bacilli were isolated from the infected tissues by the method of Prabhakaran *et al.*[21] using density gradient centrifugation of homogenized tissues on solutions of sucrose and KCL. The isolated bacilli were then used to prepare antigen using a slightly modified method of Dharmendra.[6] The *M. leprae* isolate Lot A containing a total of $1{\cdot}4 \times 10^{11}$ AFB (58% yield) and Lot B containing $6{\cdot}6 \times 10^{11}$ AFB (55% yield) were added to chloroform, and ground using a mortar and pestle. The complete evaporation of chloroform was followed by the addition of 10 ml ether. The AFB-ether mixture was centrifuged at $26{,}500 \times \boldsymbol{g}$ for 30 min at 4°C (Sorvall RC 2-B centrifuge fitted with SS-34 head, Dupont Instruments, Newton, Conn.) and the supernatant discarded. The sediment was washed again with 8 ml ether and the ether was allowed to completely evaporate from the sediment at room temperature. The dry sediment was suspended in physiological saline and autoclaved. The autoclaved suspensions were sonicated at 4°C for 10 min at 50 watts (Sonifier Cell Disruptor, Model W-185, Heat Systems Ultrasonics, Inc., Plainsview, NY) and the concentration of protein was determined by the method of Lowry *et al.*[14] using bovine serum albumin as a standard. Thirteen millilitres from Lot A contained 350 μg of protein/ml and 17·0 ml from Lot B contained 1300 μg of protein/ml. Both lots were titrated for dose responsiveness, and toxicity to mononuclear cells.

Preparation of mononuclear cell suspension

Blood was collected in heparinized vacuum tubes by cardiac puncture immediately prior to sacrificing the susceptible animals and by venipuncture of the resistant ones. A sample was removed for white blood cell (WBC) enumeration (Coulter Counter, Coulter Electronic Inc., Hialeah, FL) and differential counts after Wright-Giemsa staining. Mononuclear cells were then isolated by discontinuous density gradient centrifugation. Twenty millilitres of a 1:1 mixture of heparinized whole blood and physiological saline was layered onto 6·0 ml of Ficoll–Hypaque (Lymphoprep®, Nyegaard and Co., Oslo, Norway) and centrifuged at $400 \times \boldsymbol{g}$ for 45 min at 20°C. The mononuclear cells isolated from the Ficoll–Hypaque-plasma interface were resuspended with physiological saline to a volume of 40 ml and centrifuged at $800 \times \boldsymbol{g}$ for 15 min at 20°C. To deplete platelets, the mononuclear cells were then resuspended with saline to 40 ml and centrifuged at $250 \times \boldsymbol{g}$ for 10 min. The supernatant was discarded and the pelleted mononuclear cells were then resuspended in 3·0 ml of RPMI 1640 (Associated Biomedics Systems, Buffalo, NY), a WBC performed, and the percentages of lymphocytes and viable cells determined by the leukocyte peroxidase stain[10] and

exclusion of 0·4% w/v trypan blue in normal saline respectively. The mononuclear cell suspension was diluted to contain a final concentration of 1×10^6 viable, peroxidase negative cells per ml in RPMI-1640 supplemented to contain 20% v/v heat inactivated fetal calf serum (Pacific Biological, Bio-Rad Laboratories, Richmond, CA), penicillin (100 U/ml) and streptomycin (100 μg/ml).

Cell cultures, labelling and harvesting

Peroxidase negative, viable cells (2×10^5) were incubated for 72 hr in U-bottom polystyrene microtitration plates (Dynatech Laboratories, Inc., Alexandria, VA) in a final volume of 200 μl. The cells were exposed for various time periods to the following antigen(s) and mitogen:

1 Con-A was added in concentrations of 3·12, 0·78 and 0·19 μg per well and the cells in 4–5 replicate cultures were harvested after 72 hr of incubation.
2 Con-A plus Dharmendra antigen(s): Con-A was added in concentrations of 3·12, 0·78 and 0·19 μg per well together with a constant amount of sonicated Dharmendra antigen(s) (15 μg protein per well). The cells in 4–5 replicate cultures were harvested after 72-hr incubation.
3 Sonicated Dharmendra antigen(s): Sonicated Dharmendra antigen(s) (15 μg protein per well) was added and the cells in 6–8 replicate cultures were harvested after 3 and 7 days of incubation.

The cultures were incubated at 37°C in a 5% CO_2–95% air atmosphere with a relative humidity greater than 95%. The mitogen and antigen plus mitogen cultures were pulsed for 5–6 hr with 1 μCi of tritiated thymidine (^{3}H-thymidine, specific activity 71·8 Ci/mM, New England Nuclear, Boston MA) in 10 μl of saline. The cultures were subsequently harvested onto fibreglass filters using a mechanical cell harvester (Titertek, Flow Laboratories, Inc., McLean, VA). One hundred millilitres of NCS Tissue Solubilizer (Amersham Corp., Arlington Heights, IL) were added to moist filters in scintillation vials. After 15 min at room temperature, 6·0 ml of scintillation counting fluid (Eastman Ready-to-Use I) was added. The vials were placed into a Beckman LS-250 liquid scintillation counter for counting. The incorporation of ^{3}H-thymidine was corrected for background and quenching and results were expressed as disintegrations per minute (DPM). Results were analysed for statistical significance on a Hewlett Packard 9845B computer using the one-tailed paired *t*-test and by its non-parametric equivalent, the Wilcoxon signed rank test.[4]

Results

Preliminary experiments involved the preparation and characterization of sonicated Dharmendra antigen(s) and establishing dose–response relationships

of Con-A to determine minimal, intermediate, and optimal doses of the mitogen. To determine the degree of specificity and toxicity of sonicated Dharmendra antigen(s) Lots A and B were titrated in microculture using mononuclear cells from a healthy human contact of leprosy patients (A.B.) and a presumed leprosy naïve individual (F.B.). The results of this titration are represented in Table 1. In both Lots A and B, 15 μg of sonicated Dharmendra antigen(s) per well was optimal. After titration, these lots were pooled, aliquoted and stored at $-80°C$. Only the required amount was thawed as needed for each experiment.

A Con-A dose–blastogenic response relationship with mononuclear leuko-

Table 1. Six-day blastogenic response of mononuclear cells from healthy humans exposed to various concentrations of integral *M. leprae* and Lots A and B preparation of sonicated Dharmendra antigen(s)

Culture + antigen(s)	N*	A.B.†	SI‡	N	F.B.§	SI
None (control)	8	668·4 ± 184·5¶	1	8	979·7 ± 298·9	1
Integral *M. leprae*						
100:1**	4	1497·9 ± 73·3	2·2	4	399·8 ± 29·7	0·4
10:1	4	1162·8 ± 297·4	1·7	4	Not done	
1:1	4	433·4 ± 257·1	0·6	4	308·9 ± 23·5	0·3
Dharmendra Lot A						
30‡‡	4	2942·5 ± 806·5	4·4	3	1183·9 ± 274·3	1·2
15	4	5915·3 ± 1111·5	8·8	3	1009·4 ± 369·3	1·0
3	4	4247·9 ± 1458·0	6·4		Not done	
0·6	4	2998·2 ± 618·5	4·5	3	543·7 ± 40·7	0·6
0·12	4	1179·6 ± 481·9	1·8		Not done	
0·024	4	1003·6 ± 254·6	1·5	3	473·0 ± 28·6	0·5
Dharmendra Lot B						
30	3	3656·2 ± 1379·0	5·5	2	742·0 ± 188·6	0·8
15	4	5106·5 ± 1253·6	7·6	2	539·8 ± 68·7	0·6
3	4	3148·3 ± 122·5	4·7	3	696·6 ± 127·5	0·7

* Number of microtitre wells assayed.

† A.B. = individual in frequent contact with leprosy patients and previously titrated as a high responder to *M. leprae*.

‡ Stimulation Index $= \frac{\text{Culture + Antigen}}{\text{Control}}$.

§ F.B. = assumed leprosy naïve individual.

¶ Mean ± SD DPM's.

** Ratio of integral *M. leprae* organisms to viable lymphocytes in culture.

‡‡ Concentration of sonicated Dharmendra antigen in μg of protein/well.

cytes isolated from the peripheral blood of three normal armadillos was determined after 76-hr exposure to varying concentrations of Con-A. (Data not shown.) Doses of 0·04 μg per well were not stimulatory; 0·19 μg caused minimal stimulation and 0·78 μg moderate stimulation. The optimal dose of Con-A, with respect to ^{3}H-thymidine incorporation, was 3·125 μg per well; 6·25 μg/culture was slightly inhibitory and 12·5 μg was strongly inhibitory when compared to the response at 3·125 μg per well. For lymphocyte blastogenesis in armadillos Con-A was used at 3·125 μg as a maximum stimulatory concentration, 0·78 μg as an intermediate or mid-range stimulatory concentration and 0·19 μg as a minimal or low stimulatory concentration.

Results of the dose responsiveness of mononuclear cell cultures from resistant and susceptible armadillos to Con-A alone and the effects of the addition of sonicated Dharmendra lepromin in co-culture with Con-A are shown in Table 2. The mononuclear cell cultures from susceptible and resistant armadillos were similar, each containing 3·35 ($\pm$0·5) $\times 10^5$ (mean $\pm$ SD) and 3·32 ($\pm$1·2) $\times 10^5$

Table 2. Incorporation of ^{3}H-thymidine by armadillo peripheral blood mononuclear cells stimulated with Con-A alone or stimulated with Con-A plus sonicated Dharmendra (S. Dharmendra) lepromin

Culture	Source of cultured mononuclear cells	
	Susceptible armadillos (N = 18)	Resistant armadillos (N = 13)*
Controls	1209 $\pm$ 329†	1051 $\pm$ 256
Con-A (0·19 μg)	4518 $\pm$ 1971	5609 $\pm$ 1973
Con-A (0·19 μg) + S. Dharmendra	3771 $\pm$ 1520	2157 $\pm$ 683§
% Suppression by S. Dharmendra	16·5‡	61·5
Con-A (0·78 μg)	40,668 $\pm$ 10,064	40,192 $\pm$ 11,677
Con-A (0·78 μg) + S. Dharmendra	21,858 $\pm$ 11,642¶	16,099 $\pm$ 6688¶
% Suppression by S. Dharmendra	46·3	59·9
Con-A (3·125 μg)	113,205 $\pm$ 26,790	162,896 $\pm$ 43,650
Con-A (3·125 μg) + S. Dharmendra	153,014 $\pm$ 34,111¶	101,852 $\pm$ 34,250¶
% Suppression by S. Dharmendra	−35·2	37·5

* Multiple assays were done on each of five resistant armadillos (No. 40, N = 2; No. 199, N = 3; No. 301, N = 2; No. 321, N = 2; No. 323, N = 4).

† Mean values were calculated in each experiment based on four to five replicate cultures. These mean values were used in calculating the presented means $\pm$ SEM DPM/well.

‡ % Suppression by sonicated Dharmendra = 100 − [(DPM of Con-A + sonicated Dharmendra × 100 ÷ DPM of Con-A]

§ Significantly different from Con-A alone, $p < 0{\cdot}05$, paired *t*-test or Wilcoxon signed rank test.

¶ Significantly different from Con-A alone, $p < 0{\cdot}01$, paired *t*-test or Wilcoxon signed rank test.

mononuclear cells respectively. The monocytes (peroxidase positive cells) in culture averaged $\pm$SD 1·35 ($\pm$0·5) $\times 10^5$ for susceptible armadillos and 1·32 ($\pm$1·2) $\times 10^5$ for resistant armadillos. The percentages of viable cells averaged 98% (ranging from 95 to 100%) for both groups of armadillos.

There was no significant difference between the responses to Con-A alone of mononuclear cell cultures from susceptible and those of resistant armadillos. The

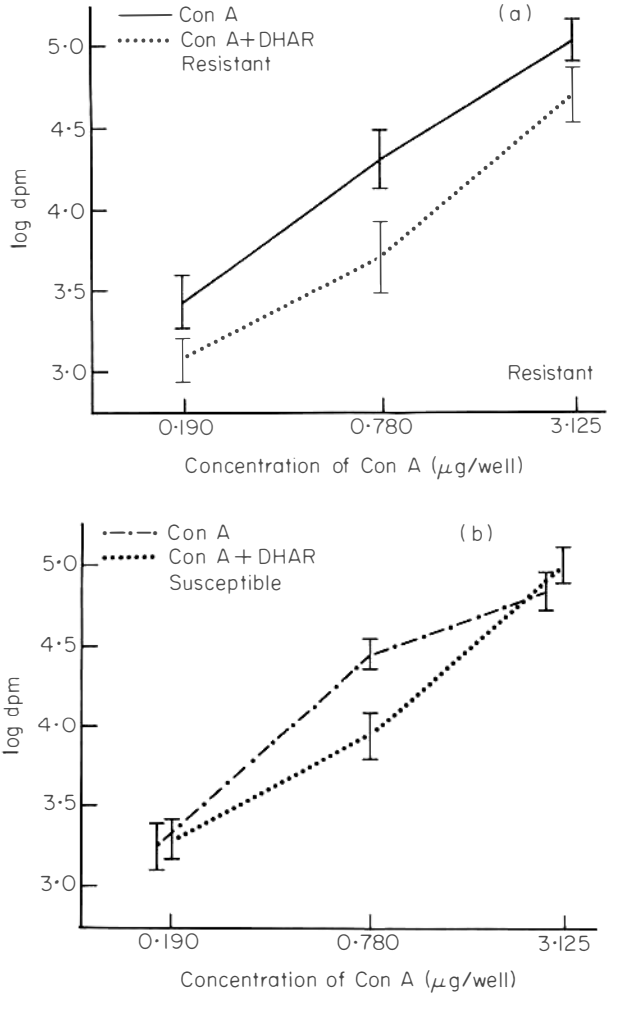

Figure 1(a). Mean $\pm$SEM DPM ^{3}H-thymidine incorporation of peripheral blood mononuclear cells from leprosy susceptible (S) armadillos after exposure for 72 hr to Concanavalin-A (Con-A) alone or concomitantly with 15 μg of sonicated Dharmendra antigen (DHAR). (b) Mean $\pm$SEM DPM ^{3}H-thymidine incorporation of peripheral blood mononuclear cells from leprosy-resistant (R) armadillos after exposure for 72 hr to Concanavalin-A (Con-A) alone or concomitantly with 15 μg of sonicated Dharmendra antigen (DHAR).

simultaneous addition of Con-A and sonicated Dharmendra lepromin had varying effects when compared to cultures receiving Con-A alone. Susceptible armadillos responded to sonicated Dharmendra lepromin by suppressing responses to intermediate doses of Con-A, enhancing responses to the highest dose of Con-A, and showing no significant change in response to the lowest concentration of Con-A. Mononuclear cell cultures from resistant armadillos responded to sonicated Dharmendra lepromin by significantly suppressing responses to all three concentrations of Con-A.

Examining the data graphically (Figure 1(a) and (b)) it may be seen that there is a consistent depression by sonicated Dharmendra lepromin in resistant animals and that the dose–response relationships of Con-A with and without sonicated Dharmendra lepromin are essentially parallel (Figure 1(a)). In susceptible armadillos, sonicated Dharmendra lepromin causes a change in the dose–response relationship resulting in no change at the low dose, suppression at the intermediate dose and enhancement of the response at the high dose of Con-A (Figure 1(b)).

Sonicated Dharmendra lepromin alone was added to other mononuclear cell cultures from these animals. In 3-day cultures there was a statistically significant ($p < 0{\cdot}025$ one-tailed paired t-test or Wilcoxon signed rank) blastogenic response in susceptible armadillos (1595 ± 416 [15], mean $\pm$ SEM [N] DPM in control, unstimulated cultures vs. 4188 ± 1573 in replicate, Dharmendra-stimulated cultures) but in resistant animals (902 ± 341 [7] in control cultures vs. 7664 ± 2897 in replicate, Dharmendra-stimulated cultures) the differences were not significant. In 7-day mononuclear cell cultures, neither the susceptible (882 ± 365 [16] in controls vs. 1360 ± 729 in replicate Dharmendra-stimulated cultures) or the resistant (450 ± 100 [13] in controls vs. 1946 ± 861 in replicate, Dharmendra-stimulated cultures) armadillos showed statistically significant stimulation by Dharmendra lepromin.

Discussion

Studies of Mehra[16, 17] have utilized a single concentration of Con-A which is given as 0·4 or 0·5 μg/well. The human Dharmendra antigen was prepared by Abe (National Institute for Leprosy Research, Tokyo, Japan) and is represented in cultures as '1:10'. [In our laboratory an aliquot from this same lot of Dharmendra antigen contained $2{\cdot}9 \times 10^6$ acid-fast bacilli ml^{-1} and 826 μg of protein ml^{-1}.] With these reagents, Mehra *et al.*[16] have shown that peripheral blood mononuclear cell cultures from some human leprosy patients show lower incorporation of ^{3}H-thymidine when exposed to both Con-A and Dharmendra lepromin than if stimulated with Con-A alone. This has been interpreted by these investigators as detecting *M. leprae* specific suppressor cells in these individuals. By these criteria, these cells were found in 32 of 35 lepromatous and 15 of 15

borderline leprosy patients, but in only 2 of 15 tuberculoid patients and 2 of 30 healthy control subjects. Later these cells were shown to belong to the TH_2^+ subset of T lymphocytes.[16]

Quite to the contrary, Nath & Singh[18] using whole, autoclaved *M. leprae* in co-culture with an optimally stimulatory concentration of Con-A, describe an antigen-generated suppression of Con-A stimulated lymphocyte transformation in 80% (17/21) of tuberculoid leprosy patients. In lepromatous patients, they describe variable results but the number of patients showing suppression was low and generally the responses were enhanced when *M. leprae* was added to lymphocyte cultures stimulated with Con-A.

The present findings in armadillos using 15 μg sonicated Dharmendra antigen prepared from armadillo grown *M. leprae*, and earlier work in humans with leprosy using 15–30 μg of Dharmendra antigen prepared by Abe, are in agreement with Nath & Singh[18]; in that the Dharmendra lepromin-induced reduction in ^{3}H-thymidine incorporation in Con-A stimulated peripheral blood mononuclear cell cultures is found in animals resistant to leprosy. In armadillos with disseminated leprosy infections, sonicated Dharmendra lepromin has variable effects depending on the dose of Con-A used to stimulate the cultures.

Clearly there are distinct differences in experimental results using co-culture techniques with *M. leprae* antigens and Con-A to test for suppressor cell phenomena in leprosy patients. These differences have been attributed to the type of *M. leprae* antigen used;[1, 18, 27] the treatment status of the patients;[18] the choice of mitogens, i.e. PHA or Con-A;[27] the final percent serum supplement in the culture medium;[27] and the duration of the *in vitro* cultures.[19] The present observations in armadillos would indicate that the concentration of the Con-A mitogen should be added to this list of variables.

Undoubtedly variations in methodology, reagents and experimental design could explain the different results obtained in different laboratories. The *in vitro* assay system is technically simple and direct, but the biological events occurring in the mononuclear cell cultures concomitantly stimulated with antigen and mitogen are clearly complex. Blastogenic responsiveness to Con-A alone *in vitro* is influenced by a variety of factors[8] and constituents of mycobacteria in general can suppress lymphocyte blastogenesis directly.[28] For example, mycobacterial D-arbino-D-mannan purified from culture filtrates of *M. tuberculosis* can suppress lymphocyte responsiveness. This suppression is not contingent upon prior exposure of individuals to *M. tuberculosis* and the generation of suppressor cells is not implicated.[7] Bjune[2] has observed that some preparations of *M. leprae* (sonicated bacilli) contain factor(s) able to suppress lymphocyte responsiveness *in vitro* in a fairly non-specific way. Furthermore, using co-culture techniques with combinations of PHA and *M. leprae* sonicate, this investigator[3] concluded that the results are 'a non-specific phenomenon not related to antigen sensitized lymphocytes'. Indeed, it is not out of the question that some of the observations made in this study could be related to a peculiarity of the Con-A system. Like all

lectins, Con-A will bind to sugars in solution. It has been shown that Con-A binds strongly to D-arabinomannan found in culture filtrates of *Mycobacterium tuberculosis*.[5] It is possible that polysaccharides derived from sonicated Dharmendra antigen when added simultaneously with Con-A, absorbed significant amounts of Con-A and reduced its effective stimulatory concentration. This could explain the shift in the Con-A dose response curve as illustrated in Figure 1(a) in resistant armadillos. However, generally 72-hr mononuclear cell cultures from resistant armadillos when first exposed to 3·16 μg of Con-A for 16 hr and then exposed to 15 μg of sonicated Dharmendra antigen, gave a response less than that in 72-hr replicate mononuclear cell cultures exposed for 56 hr to 3·16 μg of Con-A. Furthermore, 72-hr mononuclear cultures from resistant armadillos when first exposed to 15 μg of sonicated Dharmendra antigen for 16 hr and then exposed to 3·16 μg of Con-A, gave a response far less than 72-hr replicate mononuclear cell cultures exposed for 56 hr to 3·16 μg of Con-A (data not shown). Although Con-A was only tested at one concentration, the staggered addition of these two reactants still shows a suppressive effect exerted by sonicated Dharmendra antigen on mononuclear cell cultures from resistant armadillos. In susceptible armadillos, 72-hr mononuclear cell cultures first exposed to 3·16 μg of Con-A for 16 hr and then exposed to 15 μg of sonicated Dharmendra antigen, show an augmented response when compared to 72-hr replicate mononuclear cell cultures exposed for 56 hr to 3·16 μg of Con-A. Seventy-two hour mononuclear cell cultures from susceptible armadillos, when first exposed to 15 μg of sonicated Dharmendra antigen for 16 hr and then exposed to 3·16 μg of Con-A, gave a response similar to 72 hr replicate mononuclear cell cultures exposed for 56 hr to 3·16 μg of Con-A.

In short, such co-culture experiments, while appearing to be quite simple and direct, are probably influenced by a variety of factors and a variety of cells, helper T, suppressor T, effector T, antigen processing by macrophages, suppressor macrophages, suppressor B lymphocytes, etc., each responding with a variety of receptors to potentially different antigens of *M. leprae* and each T subset probably having complex dose–response relationships to Con-A. Thus, considerable reservations are in order regarding whether or not this experimental system reflects *in vivo* phenomena.

Within the limits of this system, the most striking finding in the present study is the consistent depression of responses by sonicated Dharmendra antigen in resistant armadillos at all three concentrations of Con-A. This would imply that suppressor mechanisms are operative in resistant animals under these conditions *in vitro*. We have also observed depression of responses by Dharmendra antigen and sonicated Dharmendra antigen using the same *in vitro* assay system in leprosy patients with potential resistance (tuberculoid cases) and in individuals with presumably effective immunity (lepromin positive normal individuals).[23] To the extent that these results can be extrapolated to the *in vivo* situation, these suppressor cells teleologically probably exist to protect the host against

delayed-type hypersensitivity reactions (Type I or reversal-type reactions in borderline and tuberculoid leprosy clinically).

M. leprae may activate suppressor cells *in vitro* in susceptible armadillos. One could interpret the data obtained at a dose of 0·78 μg/well of Con-A (Figure 1(a), Table 2) in this fashion. If this finding can be extrapolated to the *in vivo* situation, these suppressor cells may operate on protective cell-mediated immunity. A more likely explanation in our view on teleologic grounds, is that these suppressor cells operate to decrease antibody responses to *M. leprae* antigens and may function in preventing humoral immune responses harmful to the host, i.e. Type II or erythema nodosum leprosum reactions occurring in lepromatous leprosy clinically. The enhanced responses seen in leprous armadillos in the *in vitro* co-culture experiments at a high concentration of Con-A are in agreement with the observations of Nath & Singh[18] in human lepromatous leprosy and may represent concomitant stimulation of an expanded clone of B lymphocytes in this system. These B lymphocytes could be reacting to portions of the complex antigenic mosaic of *M. leprae* which may be the same or may be quite different from those involved in protective cell-mediated immune responses.

This technically simple system, in our view, is far too biologically complex to allow differentiation of these and probably any number of equally valid alternate possibilities.

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Skin test activity of an antigen fraction prepared from *Mycobacterium leprae* compared with standard lepromin and tuberculin PPD in leprosy patients

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Summary The reactivity of a cell wall antigen fraction, MLW1, prepared from *Mycobacterium leprae*, which induces strong lymphocyte responses *in vitro*, was compared in the lymphocyte stimulation test (LST) and the 48-hr skin test reaction in leprosy patients. A strong LST response was usually accompanied by a strong skin test response and *vice versa*. As a skin test reagent MLW1 was compared with standard lepromin and tuberculin PPD, and a significant correlation ($r = 0{\cdot}79$, $p < 0{\cdot}001$) was found between MLW1 and standard lepromin. Being a purified and highly-active preparation that can be standardized based on protein concentration, MLW1 should be considered as an alternative to lepromin in the early reaction.

Introduction

The standardization of lepromin is based on the number of acid fast bacilli/ml.[1] In contrast, the standardization of tuberculin PPD is based on functional activity assayed in experimental animals. Another disadvantage of lepromin compared to tuberculin, which has been shown in both experimental animals[2] and man[3,4], is that repeated testing leads to sensitization of the test subject.[5] A skin test reagent which does not induce sensitization and is easy to standardize, like tuberculin, would be a great improvement in leprosy.

A cell wall antigen preparation from *Mycobacterium leprae* called MLW1,

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producing only one line corresponding to *M. leprae* antigen 7 in crossed immunoelectrophoresis, induced strong *in vitro* lymphocyte responses in patients with tuberculoid leprosy and in healthy contacts of leprosy patients, while lymphocytes from lepromatous leprosy patients and non-exposed controls did not respond.[6] Like PPD, MLW1 also consists of solubilized antigens in which the amount of protein can be determined. Thus, it can be standardized on the basis of biological activity per μg protein as PPD for use as a skin test reagent. However, since MLW1 is known to contain crossreacting antigenic determinants, a marked contribution of such determinants to the skin test activity of the preparation would reduce its usefulness as a skin test reagent.

In the present study the ability of MLW1 to stimulate lymphocytes *in vitro* was compared with its skin test activity in leprosy patients. Its potency and specificity as a skin test reagent were compared with standard lepromin and tuberculin PPD.

Materials and methods

PATIENTS

All the patients in this study attended the All Africa Leprosy and Rehabilitation Training Centre (ALERT) in Addis Ababa, Ethiopia. Thirty-four patients, 16 females and 18 males, with a median age of 24 years (range 13 to 39), were included. They were all classified clinically according to the Ridley-Jopling scale,[7] and, in addition, 13 patients were also classified histologically. There were two patients with tuberculoid/borderline tuberculoid leprosy (TT/BT), 21 with BT, 6 with borderline lepromatous (BL) and 5 with lepromatous leprosy (LL). Ten of the patients had been treated with dapsone (DDS, 100 mg/day) for a period varying from 1 month to 5 years; the others were untreated.

SKIN TESTING

The following three antigens were injected intradermally on the same forearm with the injection sites at least 4 cm apart in a volume of 0·1 ml: 1 The lepromin (obtained from Dr W F Kirchheimer, Carville, Louisiana) was armadillo derived and contained $1{\cdot}6 \times 10^7$ bacilli. 2 A fractionated preparation of *M. leprae* of armadillo origin, called MLW1[6], 0·2 or 2 μg protein. 3 Tuberculin purified protein derivative (PPD) from Statens Seruminstitut, Copenhagen, Denmark, Batch RT23, 0·04 μg protein (2 TU). Reactions were read after 48 hr; the induration was measured with a ruler and the mean of the longitudinal and transverse diameter recorded. An induration of 5 mm or more was considered a positive reaction.

LYMPHOCYTE STIMULATION TEST

Blood was drawn for the LST on the same day as skin testing was performed, but prior to the injection of the antigen. Mononuclear cells were isolated and cultured as previously described.[6] Briefly, 10^5 cells/well were stimulated with 1 MLW1, the same preparation as used in skin testing, and 2 tuberculin PPD, Batch RT33, Statens Seruminstitut, and then cultured in triplicates for 6 days. Proliferation was measured as ^{3}H-thymidine incorporation. The median counts per minute (cpm) for each triplicate was used and the degree of stimulation expressed as Δcpm = cpm of stimulated culture − cpm of unstimulated control. According to our previous study[8] an individual with an LST response of Δcpm $\geqq$ 5000 was defined as a responder.

Results

The antigen preparation MLW1 was tested as a skin test reagent in patients with various clinical forms of leprosy at two doses, either 0·2 or 2 μg per skin test site. Figure 1 compares in 23 patients the capacity of MLW1 to induce a 48-hr skin reaction with its capacity to stimulate lymphocytes *in vitro* ($r = 0{\cdot}66$, $p < 0{\cdot}001$) at a concentration of 0·1 μg/ml which was shown before to give the highest median response in the BT group.[6] In 11 of the patients a positive LST response was accompanied by a positive skin test response and *vice versa*. Five patients (1

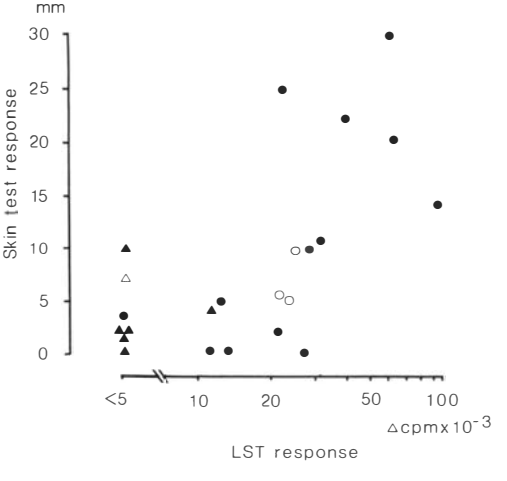

Figure 1. *In vitro* lymphocyte responses to 0·1 μg/ml and skin test responses to 0·2 μg (○, △) and 2 μg (●, ▲) of MLW1 in patients with various clinical forms of leprosy (TT/BT, n = 2; BT n = 14; ●, ○), (BL, n = 4; LL, n = 3; ▲, △). Lymphocyte stimulation is shown as net counts per minute (Δcpm = cpm of stimulated culture − cpm of unstimulated control) after incorporation of ^{3}H-thymidine, and skin test activity as diameter of induration in mm read at 48 hr.

TT/BT, 3 BT; 1 BL) with moderately strong LST responses (11,000 Δcpm to 27,000 Δcpm) showed a negative skin test response, while two patients (2 BL) with moderate skin test response (7 and 10 mm) showed very weak LST responses, 2300 Δcpm and 1500 Δcpm, respectively. In the 16 patients (2TT/BT, 13 BT, 1 BL) who were responders in the LST (Δcpm $\geqq$ 5000) the strength of the responses correlated better ($r = 0{\cdot}70$, $p < 0{\cdot}01$) than in the 13 patients (2 TT/BT, 9 BT, 1 BL, 1 LL) who showed a positive skin test response ($\geqq$ 5 mm) ($r = 0{\cdot}50$, $p < 0{\cdot}10$).

In 14 of the patients with TT/BT and BT leprosy, the skin test and LST were performed with both MLW1 and PPD, and in Figure 2(A) and (B), the potencies of these two reagents are compared in both tests. Based on the protein concentrations which were used, the ratio between the doses of MLW1 and PPD was 1:10 in the LST and 50:1 in the skin test. There were three patients who showed stronger responses to MLW1 than to PPD in the LST (Figure 2(A)), and two of these were among the seven patients (with a positive skin test response to MLW1) who showed stronger skin test responses to MLW1 than to PPD (Figure 2(B)), showing that antigenic specificity was expressed in both tests.

In Figure 3 skin test activity of standard lepromin and MLW1 is compared in patients throughout the leprosy spectrum. Fourteen of the patients were tested with a dose of 0·2 μg MLW1 per skin test site, and three of these were negative to MLW1 and positive to lepromin. The remaining 20 patients were tested with a dose of 2·0 μg MLW1, and three of these were positive to MLW1 and negative to lepromin. A dose of MLW1 between 0·2 and 2·0 μg appeared to correspond in

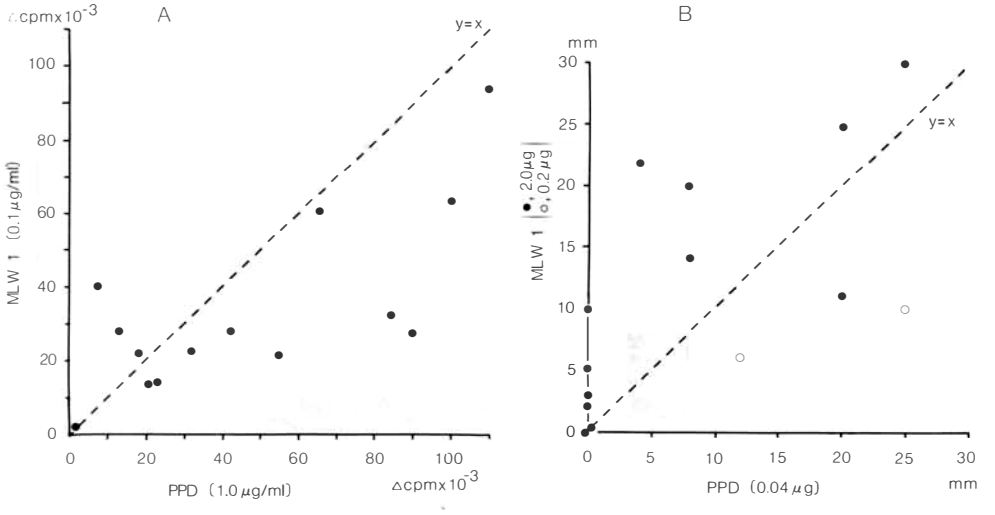

Figure 2. *In vitro* lymphocyte stimulation (A) and skin test activity (B) in 14 patients with TT/BT and BT leprosy. They were skin tested with a dose of 2 μg of MLW1 (●) except for two patients who were tested with 0·2 μg (○), while the strength of PPD was 2 TU (0·04 μg). The lines of identity, y = x, are stippled. For further explanation see legend to Figure 1.

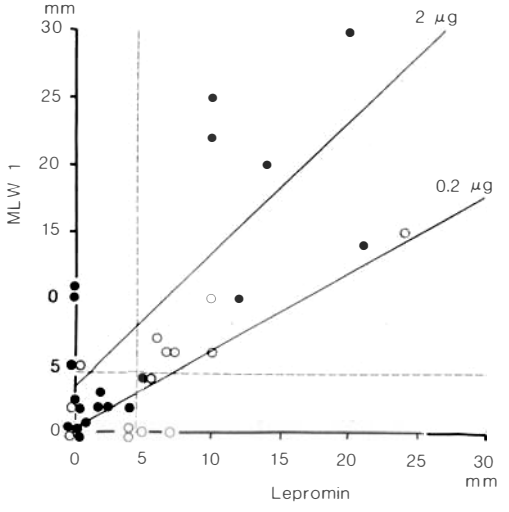

Figure 3. Correlation plot of skin test activities with MLW1 (2 μg, ●; 0·2 μg, ○) and lepromin in 34 patients with various clinical forms of leprosy (TT/BT, n = 2; BT, n = 21; BL, n = 6; LL, n = 5). The lines of regression at both doses are solid and the dividing lines between positive and negative responses are stippled. For further explanation see legend to Figure 1.

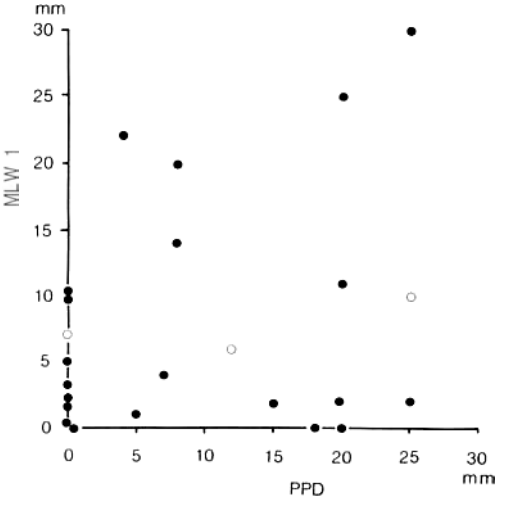

Figure 4. Correlation plot of skin test activities with MLW1 (2 μg, ●; 0·2 μg, ○) and tuberculin PPD, 2 TU, in 24 leprosy patients (TT/BT, n = 2; BT, n = 14; BL; n = 5; LL, n = 3). For further explanation see legend to Figure 1.

strength to standard lepromin. A correlation was found in skin test activity between lepromin and MLW1 ($r = 0{\cdot}79$, $p < 0{\cdot}001$).

Previously, we demonstrated[6] that tuberculin PPD and MLW1 show different antigenic specificities in the LST, and in the present study no correlation ($r = 0{\cdot}26$) was found between MLW1 and tuberculin PPD in the 24 patients tested with both antigens (Figure 4). Apparently, antigenic determinants which may be common to MLW1 and PPD did not greatly influence the skin test activity of MLW1.

Discussion

Using various antigens, skin test and LST responses have been reported by many workers to correlate well,[9–14] and soluble antigens have given better correlations than particulate antigens.[15] In leprosy, a crude antigen consisting of *M. leprae* bacilli of either human or armadillo origin has been most commonly used both in the skin test and the LST assays. Leprosin A, a skin test reagent made by Stanford *et al.*,[16] is a total sonicate of the bacilli, and since it consists of solubilized antigens, its protein content can be determined. But, in contrast to the MLW1 preparation which contains mainly one antigenic component, Leprosin A consists of various antigenic components. The MLW1 preparation has previously been shown to be a particularly potent stimulator in the LST.[6] Looking at individual responses both in leprosy patients and healthy contacts of leprosy patients, they were higher for MLW1 than for a preparation of whole *M. leprae* bacilli of human origin.[8] In the present study we have compared the potency of MLW1 to stimulate lymphocytes *in vitro* with its potency to induce an early skin reaction in patients with leprosy. It seems that the MLW1 preparation can also induce a fairly strong reaction in patients with a positive LST response. The finding of a negative skin test response in some of the patients with a moderate to strong LST response is in agreement with others who reported the LST to be more sensitive than the skin test in tuberculosis.[17–19]

Antibodies to the ML7 antigen have been shown to cross-react extensively with the BCG60 antigen,[20] and these antigens appear to be the major constituents of the MLW1 preparation and tuberculin PPD, respectively. When the LST responses to MLW1 of healthy contacts of leprosy patients and of non-exposed controls were compared, they were completely separated,[6] and all the individuals in these two groups showed higher responses to PPD than to MLW1, in contrast to the group of patients with TT and BT leprosy where one third showed higher responses to MLW1 than to PPD.[21] In spite of the extensive cross-reaction which may be expressed to a varying extent in the individual responses, these results show that MLW1 and PPD are recognized as different antigens. With regard to the comparisons of the skin test and LST responses in the present study, it seems that the different antigenic specificity of MLW1 and PPD can be expressed in the skin test results, even though the relative doses of the two antigens used in these two tests are not directly comparable.

Because of the known cross-reactivity with other mycobacterial antigens, the MLW1 preparation should not be regarded as a specific reagent in tests for DTH to *M. leprae* until it has been further tested. However, one may compensate for a certain lack of specificity by using MLW1 in combination with other antigens like tuberculin PPD and looking at the relative responses in the skin test and LST.[6] Further elucidation of its specificity is needed to evaluate its role as a prospective skin test reagent in leprosy.

Acknowledgments

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Treatment of ulnar and median nerve function loss in borderline leprosy

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Summary A rigid cortico-steroid treatment regimen was given to borderline leprosy patients who had recent nerve function loss due to reversal reaction. In order to record nerve function changes objectively a nerve index was used which was based on the results of voluntary muscle testing and sensory testing. Ninety-three ulnar and median nerves were followed in this study. Improvement in ulnar nerve function was obtained in 60% and in the median nerve in 67%. Overall improvement was better in BL nerves (85%) when compared with BT nerves (51%). The results show a more rapid recovery of median nerve function as compared to the ulnar nerve. There was a slightly better recovery of nerve function in previously untreated leprosy patients (69%) as compared to patients who developed nerve function loss while on anti-leprosy treatment (59%).

Introduction

Nerve damage in reversal reaction is a well-known complication in leprosy.[1–4] It occurs in patients in the borderline group of leprosy and may be due to a sudden change in cell-mediated reactivity, although the triggering factor is unknown.[5,6] During reversal reaction there is an inflammatory reaction at those sites where leprosybacili are found, that is in the skin and in the nerves. The inflammatory reaction in the nerves, the so-called neuritis, can result in sudden nerve damage. If this reaction is not treated quickly, then permanent nerve function loss may be the result. Drugs such as cortico-steroids should therefore be given to suppress this reaction. The drug in common use is prednisolone. The use of cortico-steroids in leprosy reactional states is not new and they have been used since the start of the use of DDS in leprosy.[7-10] The dosage,[11,12] the duration of therapy[13] and the route of administration[14] have all been a topic for discussion in the past, but there is still no collective agreement on the subject.[15] In order to assess the effect of

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cortico-steroid treatment it is necessary to measure the function of a nerve during and after treatment. For this purpose several parameters are available. In this study Sensory Testing (ST), Voluntary Muscle Testing (VMT) and Motor nerve Conduction Velocity (MCV) measurements were the techniques used to assess nerve function.

A group of patients with recent nerve damage were followed in this study during and after a fixed regimen of cortico-steroid treatment.

Materials and Methods

Thirty-six leprosy patients, twenty-four borderline tuberculoid (BT) and twelve borderline lepromatous (BL) were followed up for 1 year after they received a 6-month course of prednisolone for recent nerve function loss of the ulnar and median nerves. Patients were given 40 mg prednisolone daily for the first 2 weeks followed by 30 mg daily for the next 2 weeks. Thereafter the dosage was reduced by 5 mg every month. All patients received 100 mg DDS daily as anti-leprosy treatment. A total of 93 nerves, 53 ulnar and 40 median nerves, were followed in this study, an average of 2·6 nerves per patient.

For sensory evaluation we used the technique described by Naafs,[16] using a set of bristles with different diameters. The skin was touched with each bristle (Figure 1) in five different places. Every stimulus felt scored 2 points. Therefore the maximum sensory score for a nerve would be 50 ($5 \times 5 \times 2$) points. For muscle testing we used the tests described by Brandsma.[17] For the ulnar nerve abduction of the little finger, and for the median nerve abduction of the thumb were tested. The scoring, according to the MRC scale[18] was multiplied by a factor 10 thus also giving a maximum score of 50 for normal motor function of the nerve.

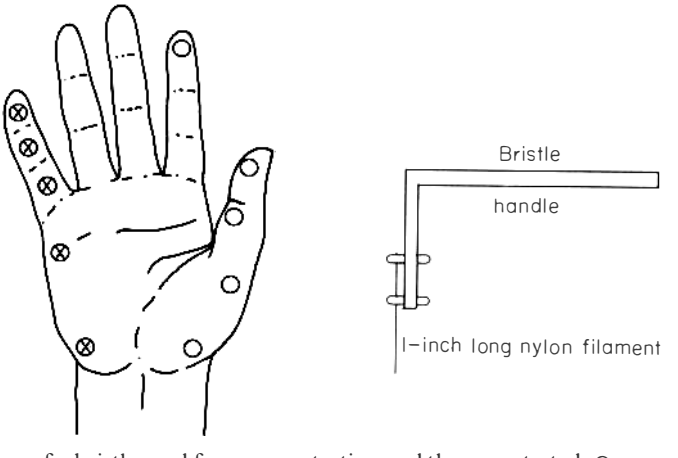

Figure 1. Diagrams of a bristle used for sensory testing and the areas tested. ○, areas for stimuli for the median nerve. ⊗, areas for stimuli for the ulnar nerve.

Motor nerve conduction velocity was assessed as described by Naafs.[19] Here also a grading scale was developed whereby the score for the conduction of the whole nerve was added to the score of the most affected segment of that nerve (Table 1).

The Nerve Index is defined as being the VMT score added to the ST score giving a score of 100 for a normal functioning nerve. In the Extended Nerve Index the MCV is added to the Nerve Index.

An increase of at least 15 points was considered an improvement, a loss of more than 15 points deterioration. A change of less than 15 points either way was regarded as *status quo*. Only patients with nerves with an initial score of 70 or less on the Nerve Index were admitted to the study. In addition the duration of nerve function loss was required to be less than 6 months.

Results

In 29 patients (80%) the nerve function improved; it remained unchanged in four patients and three patients showed deterioration of one nerve.

Thirty-two (60%) of the 53 ulnar nerves improved as did 27 (67%) of the 40 affected median nerves, thus giving a total of 59 (63%) improved nerves. Figure 2 shows the average improvement of these nerves. It will be seen that the median nerve was less affected from the start. More marked improvement was also observed in the median nerve during the first 2 months than during the following 4 months: virtually no further improvement was noted after that period. This contrasts with the ulnar nerve where initial improvement was slower, but still continued after 6 months of treatment.

Table 2 relates the recovery to classification of the patients and whether patients were already on treatment when they developed nerve function loss.

Discussion

This study is a preliminary report of a much larger trial of 133 patients and is a report on the first 36 patients who had completed a follow-up of 1 year. The trial

Table 1. Scoring for motor nerve conduction velocity

55 m/sec or more	25 points
50–54·9 m/sec	20 points
40–49·9 m/sec	15 points
30–39·9 m/sec	10 points
20–29·9 m/sec	5 points
below 20 m/sec	0 points

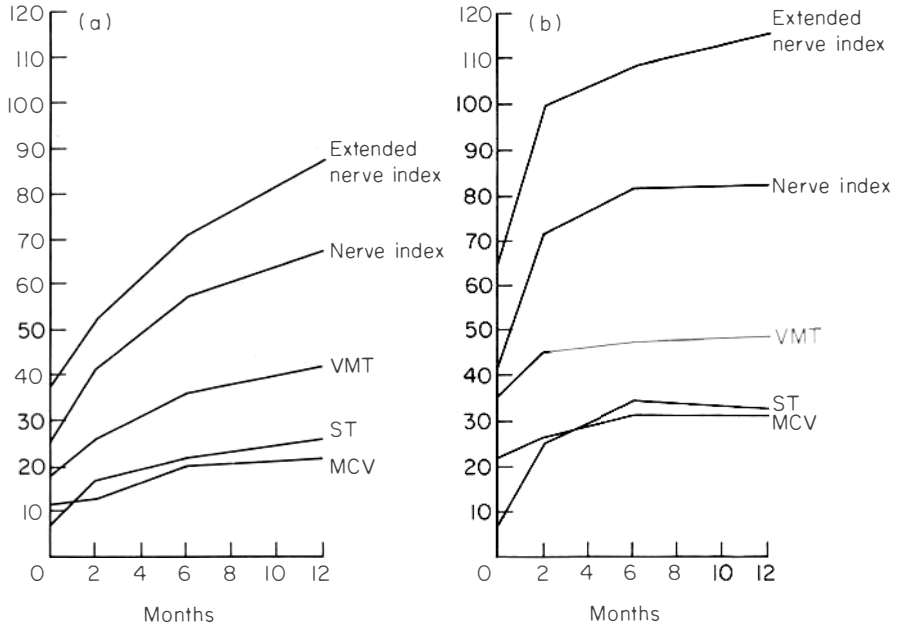

Figure 2. Improvement of (a) 32 ulnar nerves, and (b) 27 median nerves.

was designed to establish the effect of cortico-steroid treatment on neural damage during reversal reaction.[20] The anti-leprosy treatment reflects the situation prior to the introduction of multi-drug therapy as recommended by WHO. It has been suggested that cortico-steroids should be given for nerve function loss in reversal reaction at a relatively high dose for a prolonged time to be effective and to prevent recurrence.[13] These considerations led to the choice for the cortico-steroid treatment as used in this study. Side-effects can be expected at high dosages of steroids and for this reason patients were admitted to hospital during the first 2

Table 2. Nerve function recovery

	No. patients	Nerves	Improved
BT	24	59	30 (51%)
BL	12	34	29 (85%)
New patients	17	39	27 (69%)
Treated patients	19	54	32 (59%)
Total	36	93	59 (63%)

months of treatment. With this treatment no serious side-effects were encountered.

The VMT and ST are easy to reproduce and easy to perform. The idea of the Nerve Index is based on a publication by Naafs,[20] who introduced the Nerve Deficit Index as an index of the nerve function. In the present Nerve Index we have left out the scoring for pain, tenderness and enlargement of the nerve because they are subjective. In our results there is a marked difference between the rates of recovery of the ulnar and median nerves. One explanation might be the difference in anatomical level of nerve involvement. The difference can certainly also be explained by the initial higher VMT score of the median nerve.

When the study was started it was discussed whether the classification of the patient or prior treatment were important to the patient's response to treatment for recent nerve damage. Table 2 shows that BL patients responded more favourably to the treatment than BT patients. The number of BL patients, however, is small and nerve function loss in these patients may recur. A larger observation group and a longer observation time may show more definite results. There were no major differences between treated and untreated patients.

MCV is useful in a trial like this as another means of objectively assessing nerve function but for the follow-up of nerve function in treatment trials it is not absolutely necessary. No new prognostic indications are added by this method.

The Nerve Index gives a good indication of the nerve function in a leprosy patient as it gives a good picture of the changes of nerve function in treatment trials and is also valuable in the follow-up of nerve function in individual patients.

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The occurrence of leprosy in an eight-member family—a case report

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Summary A family of 6 children aged between 11 years and 11 months and their parents affected with leprosy is reported. The mother and 5 children had multibacillary leprosy and one child had paucibacillary leprosy. The father, though apparently clinically normal, showed acid-fast bacilli in the skin. This family illustrates that the incubation period of leprosy can be shorter than 1 year and the possibility that this may be related, in some cases, to intra-uterine transmission is discussed.

Leprosy is known to occur in more than one member of a family, but it is unusual to see all 6 children and both parents affected by leprosy at the same time. This paper describes the clinical, bacteriological and histopathological findings in such a family.

Case reports

1 *AL card no. SJO 7346, a female child born in 1974*, hailing from Ambo, Shoa Region, was brought to ALERT hospital on 21 August 1981 with multiple shiny nodules all over the face, ears, limbs and buttocks of 2 years' duration. The lesions increased 4 months before admission. The trunk was free of lesions.

There was no thickening of nerves or anaesthesia. A diagnosis of lepromatous leprosy of the histoid type was made. Skin smears revealed a Bacteriological Index (BI) of 4·8 and a Morphological Index (MI) of 7·8. The child was admitted and a course of rifampicin 300 mg daily for 3 weeks and dapsone (DDS) 100 mg in his neighbourhood. His present wife, who is the mother of all the children in this report, was his third. He had had children by his first and second marriages, but no medical details were available. The child described above was the third child of the third wife and he was asked to bring the others for examination, daily was given.

2 *The father accompanying the child* was examined and found to have no clinical evidence of leprosy. He was unaware of any other cases of leprosy in the family or

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together with the wife. On a subsequent attendance, he himself was re-examined and again found to be free of clinical signs, but to have a positive smear for acid-fast bacilli (AFB) at one site, namely the forehead, where the BI was 2+; it was negative at other routine sites. This positive finding was checked carefully and confirmed, and in view of the circumstances, he was treated with DDS 100 mg daily.

3 *BL card no. SJO 7475, a 10-year-old female child*, the second child of the family, had multiple erythematous and oedematous papules and plaques on the face, limbs and buttock of 3 months' duration. There was no nerve thickening or anaesthesia. A diagnosis of borderline lepromatous leprosy in reaction was made. Smears showed BI 2·5 (maximum 4) and MI 2. A skin biopsy showed that the epidermis was spongiotic, with oedema of the dermis. Below the epidermis there was a narrow free zone without any infiltrate. Below that there was a diffuse infiltrate of histiocytes, together with a few epithelioid cells and lymphocytes. AFB 3+. Conclusion: BL leprosy in reaction. The lepromin test was negative. The child was treated with rifampicin 600 mg daily for 3 weeks and DDS 100 mg daily. She was also given chloroqine for 2 weeks. The erythema and oedema subsided.

4 *DL card no. SJO 7474, a boy aged 3 years*, the fifth child of the family, was also brought on 17 February 1982 with multiple papules and plaques on the face and limbs and hypopigmented macules on the trunk of three months' duration. The lesions were fewer than in case no. 3. A diagnosis of BL leprosy was made and smear examination showed BI 2 (maximum 3). Skin biopsy showed that in the upper dermis there was a band-like infiltrate of macrophages and lymphocytes. AFB 3 to 4+. Conclusion: BL leprosy. Lepromin test was negative. The child was given rifampicin 300 mg daily for 3 weeks and DDS 50 mg daily.

5 *CL, 43-year-old female, the mother of the children, card no. SJO 7476*, had a few ill-defined hypopigmented macules on the arms, thigh and left leg. The BI was 3 (maximum 4). Biopsy showed multiple small collections of macrophages and few lymphocytes in the upper dermis. AFB 5+. Lepromin test was negative. BL leprosy was diagnosed. She was also given rifampicin 600 mg daily and DDS 100 mg daily.

6 *JL card no. SJO 7482, the youngest child in the family*, an 11-month-old boy. There were 7 small, slightly raised lesions varying from 5 to 10 mm in size on the trunk, thigh and ankle. Smears were taken from 3 of these lesions and the average BI was 3+. A biopsy of one of the lesions showed an infiltrate in the upper and mid-dermis composed of histiocytes, macrophages holding AFB, many lymphocytes and few epithelioid cells. AFB 3 to 4+, including solids. Conclusion: BL leprosy. Lepromin test was negative. The child was given rifampicin 150 mg daily for 3 weeks and DDS 25 mg daily. Considerable care was taken to assess and record this child's age as accurately as possible. Our enquiries, and the physical size and appearance, strongly suggested an age of 11 months.

7 *TML card no. SJO 7509, an 11-year-old boy*, the eldest child of the family, had

several hypopigmented macules distributed asymmetrically on the limbs and buttock. Smears showed BI 4+ and MI 3+. Biopsy revealed an infiltrate of histiocytes with a few epithelioid cells, and lymphocytes. AFB 4+. Conclusion: BL leprosy. Lepromin test was negative. The child was given rifampicin 600 mg daily for 3 weeks and DDS 100 mg daily.

8 *AL card no. SJO 7510, a 7-year-old boy*, the fourth child in the family, was also examined. He had multiple, small hypopigmented, slightly raised, oval well-defined lesions on the trunk, buttock and limbs distributed asymmetrically. Smears were negative. Biopsy revealed multiple localized collections of epithelioid cells with numerous Langhans giant cells and lymphocytes. No AFB. Conclusion: BT leprosy. Lepromin test was positive (induration 8 mm). This child was also given rifampicin 300 mg daily for 3 weeks and DDS 50 mg daily.

Discussion

In this family the mother and all the 6 children have leprosy. Out of these, only one child has paucibacillary leprosy and all the others have multibacillary leprosy. The father, though apparently clinically normal, has BI 2+ at one site. Thus all the children and probably both parents are affected with leprosy. The oldest child was 11 years and the youngest was 11 months when the disease was detected. In this family it is difficult to say who got the infection first, but the mother might have been the source of infection as her lesions were vague and difficult to detect and the skin smears were positive. Since the age difference between the children is small, they are closely associated in the family environment and the infection might have spread easily from one to the other. However, it is very unusual to find all children in one family affected with the disease. It is well known to leprologists that the leprosy bacillus does not produce the disease in all human beings with whom it comes in contact. A variety of factors have been identified to explain this supposed variation in susceptibility. These include diet, climate and incidence of other infections and other factors described as innate, inborn, constitutional, familial or hereditary.[1,2] Heredity may play an important role in the aetiopathogenesis of leprosy. Available data on the genetic factors in leprosy are still conflicting and inconclusive.[3] De Vries *et al.*[4] showed that siblings with the same type of leprosy show a significant excess of identical HLA haplotypes. Genetic studies of this family will be reported separately.

It is noteworthy that most of the cases in this family were detected by 'active' clinical examination; neither the patients nor the parents having complained about the disease. The asymptomatic hypopigmented macules were obviously not considered as an indication of leprosy (or any other disease)—a matter of particular concern, since such cases may of course be a great source of infection in the community.

In view of the finding of a positive slit skin smear at one site in the father, together with the extremely heavy family involvement, it was considered wise to

regard his as a case of leprosy and to treat with DDS. Although normal clinically, one must bear in mind the possibility that leprosy bacilli can enter the skin without producing any visible sign of the disease. Ghosh[6] reported 12 contacts showing histological changes in the dermis, including nerves containing AFB, but without any visible signs of leprosy. It is not sure whether the father is in the incubation period of the disease, or whether his immunity, aided by chemotherapy, will cope with the challenge. He remains under close clinical observation.

Almost certainly, the most interesting case in this family was the child who was 11 months old on diagnosis. Although the average incubation period is accepted as being from 2 to 5 years, it may vary from 3 months to 40 years.[7] There are very few documented cases of leprosy under the age of 1 year except those reported by Chakrabarthi[8] but the whole subject is of great current interest in view of the possibility that leprosy may, in some instances, be transmitted by the placental route. Melsom *et al.*[9] have described immunological findings which point to intra-uterine infection in leprosy, and more recently Duncan *et al.*[10] have reported the clinical and immunological findings in 4 babies of mothers with lepromatous leprosy, 2 of whom developed leprosy in infancy. Lesions were first observed at the age of 12 and 17 months respectively.

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I wish to express my sincere thanks to the Director of Hospital Services, ALERT for allowing me to publish this paper.

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HLA-D identity in a family with multiple cases of multibacillary leprosy

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Summary HLA-D identity was determined by mixed lymphocyte reaction in a family with eight members affected by leprosy. The mother (BL) and all the children (1 LL, 4BL and 1BT) were HLA-D identical, whereas the father was HLA-D identical with four of the children except two BL cases. All the children formed HLA-D identical sib-pairs with the exception of one pair (LL and BL). Analysis of disease susceptibility frequency showed the mode of inheritance to be recessive, with the probability that the affected sibs would share both haplotypes.

Introduction

Family clustering of leprosy patients is known to occur, however it is unusual to see both parents and all six children affected with leprosy at the same time.[1] The occurrence of such a family offers a unique opportunity to study the development of a disease pattern in a sample of the population that may share both common environmental exposure and possibly shared genetic susceptibility.

Recent studies have shown that the HLA system contained genes that predispose to tuberculoid leprosy by analysing the segregation pattern of parental haplotypes as observed in affected children.[2] The observations were originally made in Surinam, but were later confirmed in India.[3] In addition to these, a significantly increased frequency of HLA-DR2 antigen was observed among tuberculoid leprosy patients in multiple case family studies in India. However, a consistent association between the lepromatous leprosy and the HLA system is lacking. In this paper we report the occurrence of HLA-D identity in a mother and her children affected with multibacillary leprosy.

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Patients and methods

PATIENTS

The family studied came from the Ambo region in the Shoa Administrative Region in central Ethiopia, a distance of about 260 km from Addis Ababa from an area referred to as 'the lepromatous corner' by leprosy field workers because of the high incidence of lepromatous leprosy in the area (unpublished observations).

The patients were classified by one of us (PAS) according to the Ridley–Jopling scale.[4] The clinical diagnosis was supported by skin smear bacilloscopy for acid-fast bacilli, and skin biopsies taken from representative lesions. Furthermore, skin testing with lepromin and lymphocyte transformation test (LTT) to *M. leprae* confirmed the diagnosis and classification.

MIXED LYMPHOCYTE REACTION (MLR) AND LYMPHOCYTE TRANSFORMATION TEST (LTT)

Lymphocytes were separated from defibrinated blood on Ficol–Isopaque, gradient.[5] All cultures of lymphocytes were made in triplicate in 96-well round-bottomed trays (Cooke laboratory products, Alexandria, Va.) in 200 μl of RPMI 1640 medium containing 20% pooled, heat inactivated, normal human serum, 2 mmol/l L-glutamine and antibiotics. All cultures were incubated at 37°C in 5% CO_2—humid air, and the cells were labelled on the appropriate day with 1 μ Ci ^{3}H-thymidine per well and harvested 20 hr later with a multiple cell culture harvester (Skatron, Lierbylen, Norway), ^{3}H-thymidine incorporation was determined by scintillation counting (LKB, Bromma, Sweden).

HLA-D identity was determined by mixed lymphocyte culture and was with standard methods.[6] Briefly 5×10^5 stimulator cells in 100 μl (treated with 0·25 mg 1 ml Mitomycin C) were added to 5×10^5 responder cells in 100 μl of medium in a microtitre well. All tests were carried out in triplicate and labelled with ^{3}H-thymidine after 5 days.

The lymphocyte transformation response to *M. leprae* was performed by adding 25 μl of a 10^6 bacilli/ml preparation to 2×10^5 lymphocytes in 200 μl of medium per well in triplicates. Cells were labelled with ^{3}H-thymidine on day 5 of culture.

The stimulation index (SI) and the increase in counts/min above control (cells cultured without antigen), i.e. the CPM were calculated from the mean of the triplicate cultures. An average SI >2 was considered to be positive for the mixed lymphocytes reaction and an SI >3 for the lymphocyte transformation response.

ESTIMATION OF DISEASE SUSCEPTIBILITY, GENE FREQUENCY AND INHERITANCE PATTERN

The technique for studying the inheritance pattern of HLA haplotypes in family members affected by a disorder has been described. The basic assumption in the technique is that there is a disease susceptibility locus effectively within the HLA region. Assuming that the parents carry four different HLA haplotypes between them and these are used as markers, one can then calculate the likelihood of getting a sib-pair with the disease for each possible disease and haplotype combination of the parents. Thus, for each parental arrangement, the probabilities of the affected sib-pairs having both, one or no HLA haplotypes in common can be calculated.

Results

Patient classification and LTT response to M. leprae *antigen*

Table 1 gives clinical data and the LTT response to *M. leprae*. Five of the children and the mother had multibacillary leprosy as shown by bacilloscopy, whereas one child was paucibacillary. The father had a BI of 2, however clinical and histopathological examination revealed no signs of leprosy. Histopathological classification revealed that four of the children and the mother had BL leprosy

Table 1. Clinical data and lymphocyte transformation test (LTT) result in patients under study

			Bacilloscopy					
Initials	Age	Sex	BI	MI	Clin* DX	Hist† DX	Lepromin	LTT¶
Cha‡	33	F	3	0	BL	BL	Neg	0 (<1)
Leg§	42	M	2	—	?L	Normal	10 mm	26·1 (5·1)
Tes	12	M	4	3	BL	BL	Neg	0 (<1)
Ahe	11	F	4·8	7·8	LL (H)	LL	Neg	0 (<1)
Biz	10	F	2·5	2	BL	BL	Neg	0 (<1)
Ale	7	M	0	0	BT	BT	8 mm	56·7 (13·3)
Dir	3	M	2	—	BL	BL	Neg	0 (<1)
Jil	1	M	3	2	?L	BL	Neg	0 (<1)

* Clinical diagnosis.
† Histological diagnosis.
‡ Mother.
§ Father.
¶ $\Delta CPM \times 10^{-3}$ (SI).

whereas one child had LL leprosy. All multibacillary patients were anergic in the lepromin test. The mother who was in reaction at the time of examination had a good LTT response like the BT child and the father, whereas all the multibacillary children had no *in vitro* response to *M. leprae* antigen.

MLR and HLA-D identity

The results of the MLR in the family studied are presented in Table 2. An extramaternal child who is free from leprosy is included in this study. As shown in this table all children are HLA-D identical with the mother. However, two of the multibacillary children (Dir and Jil) are HLA-D identical with the father whereas all the rest are non-identical. The extramaternal child of the father was found to be HLA-D non-identical to all family members except the father.

DISEASE SUSCEPTIBILITY FREQUENCY

The multibacillary children in this family form 10 sib-pairs. However, one sib-pair is HLA-D non-identical. Thus the observed value for sharing haplotypes bearing HLA-D linked disease susceptibility is 0·9 (expected value 0·907). This is

Table 2. Results of determination of HLA-D-identity by mixed lymphocyte reaction

	Leg	Tes	Ahe	Biz	Ala	Dir	Jil	Ger‡
Cha	NID*	ID†	ID	ID	ID	ID	ID	NID
	Leg	NID	NID	NID	NID	ID	ID	ID
		Tes	NID	ID	ID	ID	ID	NID
			Ahe	ID	ID	ID	ID	NID
				Biz	ID	ID	ID	NID
					Ale	ID	ID	NID
						Dir	ID	NID
							Jil	NID

* HLA-D non-identical.
† HLA-D identical.
‡ Extramaternal child.

quite consistent with a recessive mode of inheritance and 'a disease gene' with a frequency (pD) of 0·05, with the probability that all affected sibs share both haplotypes.

Discussion

The results of this study show that the mother and children affected with multibacillary leprosy, with the exception of one lepromatous sib-pair, are HLA-D identical. However, studies done in the past have shown significant association between the occurrence of HLA-DR2 antigen in tuberculoid case children of non-affected parents,[3] whereas there is only one report of weak association between lepromatous leprosy with HLA-D antigen.[8] Furthermore, it was shown that normal people, HLA-D identical with lepromatous siblings, do not share the siblings specific unresponsiveness to *M. leprae*.[9]

The appearance of leprosy in mother and all six children born to her is unusual. Apart from a common environment, families may share certain genetic susceptibilities and this study may shed some light on the possible involvement of genetic factors under the special circumstances of the family studied. Furthermore, this study suggests that the HLA-D related disease susceptibility in the family studied favours a recessive mode of inheritance. However, with the small sample size a dominant mode of inheritance cannot be completely ruled out although the results are much more compatible with a recessive mode of inheritance, due to a high frequency of sharing HLA-D haplotype in the affected sibs. It must also be pointed out that the data presented in this paper are not adequate to reach any firm conclusions about the mode of inheritance because of the small sample size and possible methodological issues.[10] It will be of interest to obtain further HLA-D data in similar families if the suspected recessive mode of inheritance for familial lepromatous leprosy can be confirmed.

If sufficient numbers of affected siblings are available this method provides a very powerful test for discriminating between dominant and recessive modes of inheritance of 'disease susceptibility genes' provided the frequencies of these genes are small.[11]

Appropriate data from other HLA-leprosy associated studies should provide very useful information for this test. If the method developed[2] for analysis of non-random segregation among sibships of different sizes is combined with the method applied in this study a much more meaningful synthesis could be made from such studies.

Previous studies indicate that lepromatous leprosy occurs in individuals in which the frequency of HLA-D or other genetic markers are not increased. This suggests that the disease may occur in individuals with a normal capacity for immune response to *M. leprae* but who, due to various reasons, have subsequently become unresponsive to *M. leprae* with ensuing development of

multibacillary disease.[9] However, the occurrence of lepromatous leprosy in almost all children born to a lepromatous mother and occurring uncharacteristically at a much younger age may be influenced by genes in the HLA-D region or in linkage disequilibrium with the region. If this prediction is verified by further studies, then a method for detecting a certain proportion of pre-lepromatous individuals will be available.

Acknowledgments

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Leprosy care through traditional healers

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Summary An encouraging increase in the knowledge of leprosy has been found in traditional healers after training has been given. Evaluation was by pre- and post-training questionnaires to which scores were assigned. Their role in leprosy care at the community level is also being discussed.

The traditional healer is part and parcel of the social milieu where he commands respect and has an intimate relationship with his clientele. Considering the present manpower in the National Leprosy Control Programme we can think of this alternative approach as a boost. In addition, the negative aspects of health care as practised by many practitioners can also be corrected by the appropriate training.

Introduction

Leprosy has been given top priority in our National Programme, and by AD 2000 the Government of India hopes to eradicate leprosy.

Our grassroot level worker is a non-medical assistant who perhaps is not acceptable to the community because people have great faith in a large number of other health providers, most of whom are traditional healers. A traditional healer is any practitioner not institutionally qualified but practising any system of medicine, folk medicine and or magico-religious practices in the same socio-cultural setting as the clientele. We have been working with the traditional healers for quite some time in this area.

The present study was conducted to find out if the traditional healers are interested in acquiring modern knowledge of leprosy and other skin diseases and to see if additional training could improve the standard of their health provision.

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Materials and methods

A training programme of the traditional healers was taken up in the village Narainpur of Chiraigaon block of the Varanasi District, population 2000.

A list of traditional healers working in the area was made. Out of 122 practitioners working in the area, 20 were selected for training by using the following criteria. (1) Skin practitioners who are involved in healing skin diseases and are willing to undergo training. (2) Willing practitioners with sufficient learning capacity. (3) Those with a large client record. (4) Those practitioners with a larger catchment area. (5) Level of interest for undergoing the training.

Along with leprosy some other common skin diseases, namely, scabies, ringworm (dermatophytoses), eczema, vitiligo, pyodermas (impetigo and furunculosis) and urticaria were also taught to the trainees.

The knowledge and practice of the trainees regarding the above conditions were assessed with the help of a predesigned and pretested proforma. The same proforma was also used for post-training assessment.

The training was given on 5 consecutive days. The programme was arranged to suit the convenience of the trainees. The training material included a manual in the regional language (Hindi), photos and slides of the cases, clinical demonstration of the patients and the drugs used for treatment.

Knowledge about the causation, mode of transmission, the cardinal signs and curability of leprosy was given. The trainees were particularly told about the misconceptions prevalent in the community regarding leprosy. They were also told that dapsone is the treatment of choice and has to be continued for long periods. They were taught that the patients with any one of the cardinal signs of leprosy should be referred to the nearest leprosy clinic. They were, however, not advised to stop any other treatment that they have been practising. The post-training assessment was done by following the practitioners 1 week after the training and then at monthly intervals for 3 occasions. Retraining at individual level was also done during this follow-up period. Some patients under the treatment of trainees were also cross-checked to assess the post-training practice.

The knowledge of the practitioners before and after training was scored by giving points according to the importance of the messages, and half of the actual score was allocated to the answers given after probing. A leprosy pretesting questionnaire carried 32 points. Only those questions which carried positive scores are listed below, others are omitted.

Results

Out of the 20 practitioners, 15 (75%) were literate and 5 (25%) were illiterate and 80% of the practitioners were male while 20% were females.

Questionnaire

		Scores assigned
1	How do you recognize leprosy?	
	i Hypopigmented patches without itching.	2
	ii No pain sensation over the patch.	2
	iii Loss of hair over the patch.	1
	iv Loss of sensation of hands and feet.	2
	v Loss of eyebrows.	1
	vi Thickening of ear.	1
	vii Depressed nose.	1
	viii Deformities of hands and feet.	1
2	What is the cause of leprosy?	
	i By a germ.	2
3	Does it spread? Yes; If yes, how does it spread?	2
	i By close contact.	2
4	Can the spread of the disease be prevented? Yes, If yes, how?	1
	i Early treatment of the patient.	2
	ii Avoid contact with the patient when he is infective.	1
5	What is the treatment?	
	i Name of the medicine—dapsone.	3
	ii Dose of the medicine—100 mg daily.	2
	iii Duration of treatment—5 or more years.	2
6	When should you refer a case to a doctor?	
	i After diagnosis.	1
	ii Suspected cases.	1
	iii After 5 years of treatment.	1
7	To whom should you refer the case: RHC/Doctor/Chiraigaon PHC/Ashapur Leprosy Hospital or others (specify). One score each for any two places only.	2
		32

The age of the trainees varied from 22 to 72 years. Most of the practitioners (45%) were in the age group of 22–34 years followed by 35% in the age group of 45 years and above, while 20% were in the age range of 35–44 years (Table 1).

Out of the maximum possible score of 32, the pre-training score of practitioners varied from 2·0 to 16·0 with a mean $7{\cdot}2 \pm 4{\cdot}1$, which was 22·5% (Table 2). After training the mean score increased to $21{\cdot}6 \pm 5{\cdot}0$ which was 67·5%, thereby showing an increase of 14·4 points or 45·0% after training. The difference was found highly significant statistically ($t = 9{\cdot}83$; $P < 0{\cdot}001$).

The mean score was found to be increased during the first follow-up, i.e. after 1 month of training. It was $25{\cdot}5 \pm 5{\cdot}1$ which was 3·9 points more than the previous one. After 3 months, i.e. after 2 more follow-ups, the mean score reached to $27{\cdot}5 \pm 3{\cdot}5$, i.e. 85·3%, and the range of score was found to vary from 21·0 to 32·0, i.e. up to 100·00% in some cases. The differences in mean score in follow-ups increased significantly ($t = 3{\cdot}52$; $P < 0{\cdot}01$ and $t = 2{\cdot}49$; $P < 0{\cdot}05$), thus showing

Table 1. Age and sex distribution of selected practitioners

Age (years)	Male	Female	Total No.	Total (%)
22–34	7	2	9	45·0
35–44	3	1	4	20·0
45 and above	6	1	7	35·0
Total	16 (80·0%)	4 (20·0%)	20	100·00

Table 2. Pre-training and post-training scores of knowledge of the practitioners regarding leprosy

Period score (total = 32)	Range	Mean ± SD	(%)	*t* value (df = 18)
Pre-training	2·0–16·0	7·2 ± 4·1	22·5	
Post-training (after 1 week)	9·5–29·0	21·6 ± 5·0	67·5	$t = 9{\cdot}83$ $P < 0{\cdot}001$
First follow-up (after 1 month)	13·0–31·5	25·5 ± 5·1	79·7	$t = 3{\cdot}52$ $P < 0{\cdot}01$
Third follow-up (after 3 months)	21·0–32·0	27·5 ± 3·5	85·3	$t = 2{\cdot}49$ $P < 0{\cdot}05$

that subsequent retraining increased the knowledge of the practitioners still further.

The educational status of practitioners was an important factor in their ability to grasp the training and improve their score. The practitioners with high school or above education scored more than those with little or no education. The age and better retraining score of the practitioners did not affect their scores.

During the 3-month period of post-training assessment 12 leprosy cases attended the Rural Health Training Centre (RHTC). Out of these, 11 cases were referred by trainee practitioners and only 1 by a fellow patient who had learnt about leprosy from one of the practitioners. These leprosy cases were of various clinical types as shown in Table 3.

None of the cases were deformed and a skin smear was positive in the one lepromatous case.

When interviewed, 17 of the practitioners were also willing to be trained similarly in other diseases.

Table 3. Clinical types of the leprosy cases who attended RHTC during post-training assessment periods

Clinical type	Reported by practitioner	Referred by other persons	Total
1 Polyneuritic	1	x x	1
2 Maculo Anaesthetic	3	x x	3
3 Tuberculoid	5	1	6
4 Borderline	1	x x	1
5 Lepromatous	1	x x	1
Total	11	1	12

Discussion

The healer:population ratio is 71:1000 for the country[1] and they constitute the potential reservoir of health manpower, and we should examine the feasibility of harnessing this enormous source of traditional healers to modern and more appropriate medicine. It is estimated that the traditional healers generate primary medical care services 8 times the value of patient's visits offered by the organized Government health system.[2]

The findings of this short-period action research study are encouraging. By subsequent training and retraining the traditional healers' knowledge of leprosy was enhanced (Table 2). From a very poor pre-training level, where none of the practitioners scored above 50% after 3 months of follow-ups the level was much increased, all of the practitioners scored more than 65% and a few 100%.

The diffusion of knowledge imparted to the traditional healers and its positive effect on the community is evident from the fact that almost all of the leprosy cases who attended RHTC were referred by the trainees. Most of these cases were new, with early symptoms and were free from deformities. Interrogation revealed that most of these cases had been actively searched for by the practitioner.

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'Neural histoid'. Histoid leproma in peripheral nerve; a case report.

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Summary A case of 'neural histoid' nodules occurring in the peripheral and cutaneous nerves, in an otherwise inactive case of lepromatous leprosy is presented. It is postulated that this is an instance of relapse of the disease occurring exclusively in the nerve, the nodules resulting from the unrestricted multiplication of drug-resistant mutants. The pathogenesis of the condition is discussed.

Introduction

In 1963 Wade described a variant of the conventional nodular lepromatous leprosy to which he gave the name 'Histoid' variety of lepromatous leprosy.[1] Clinically he identified three types of lesions—the 'cutaneous' and the 'subcutaneous' histoids occurring in the cutis and the subcutis; and the 'sharply delimited plaques, on the points of pressure'. Several publications that followed confirmed Wade's observations regarding the occurrence of these lesions and in the locations mentioned by him.

A lepromatous case who had been inactive for over 9 years and on continuous dapsone monotherapy was referred from the Department of Epidemiology, SLR & TC, for an opinion regarding the small lumps that had arisen over the radial cutaneous nerves. Clinically the lumps were suggestive of nerve abscesses. One of the lumps on histopathological examination proved to be a histoid nodule in the nerve. In view of this interesting finding and the paucity of reports on histoid lesions in the nerve, the details of the case along with the investigations and findings are presented here.

Case report

Patient S, male, aged 27 years, was admitted at the SLR & TC in January 1969 as a

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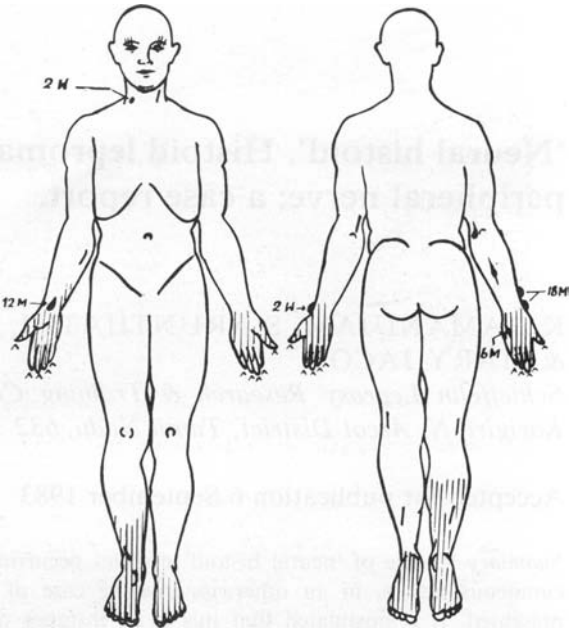

Figure 1. Chart showing the clinical condition of the patient, 7 October 1982.

lepromatous case with recurrent reactive episodes. His BI was 3·75. Initially he was treated with antimalarials and antimonials and later with clofazimine. Dapsone therapy was then started, reaching up to 100 mg per day. Attacks of neuritis continued to occur. The dose of dapsone was reduced to 50 mg per day and continued regularly. He became bacteriologically negative in May 1973. Subsequently, he developed neuritis on two occasions. In course of time ENL and neuritis abated. Dapsone was continued. The skin smears remained negative.

In October 1982, he was referred to us for an opinion of the small swellings over the radial cutaneous nerves. Clinical examination showed that he was an inactive case of lepromatous leprosy with marked thickening of several peripheral and cutaneous nerves and anaesthesia over the hands and feet (Figure 1). The nerves were firm and non-tender with nodular swellings over some of them (Figure 2). Some of the nodules were of 6–18 months' duration and the others had been present for 2 weeks. The latter were erythematous and tender. There was a suggestion of fluctuation over one of the swellings. A tentative diagnosis of '?nerve abscess' was made and the case was referred to a surgeon for exploration and biopsy of one of the nodules. Skin smears were negative.

The first biopsy was taken from the pearly nodule over the thickened right posterior antebrachial nerve and sent for histopathological examination with a clinical diagnosis of '?neuroma ?histoid nodule'. The report came as 'histoid nodule, nerve'. This unexpected finding stimulated further interest in the case and

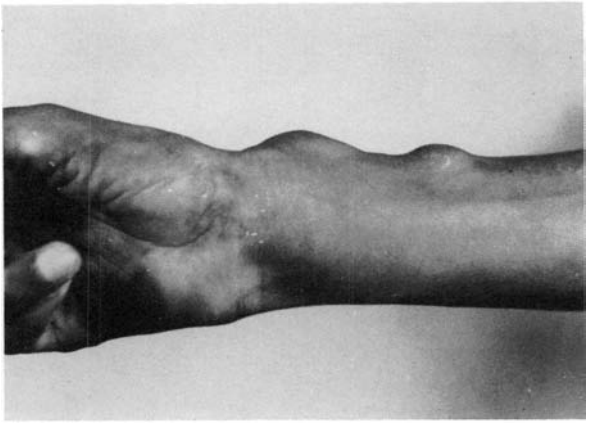

Figure 2. Large swellings in the region of the right wrist.

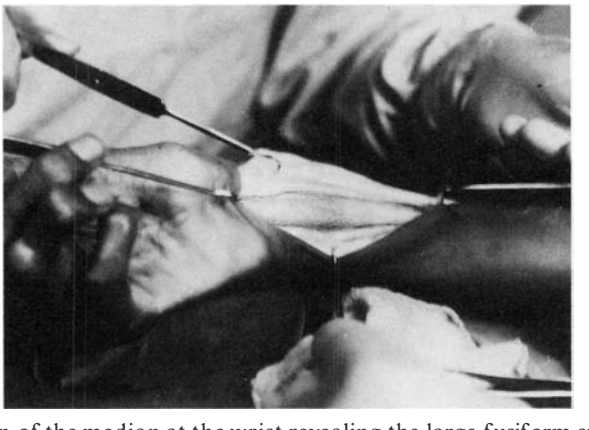

Figure 3. Exploration of the median at the wrist revealing the large fusiform swelling in the nerve prior to its entry into the carpal tunnel.

led to the exploration, biopsy and histological examination of the nodule over the right radial cutaneous and the large, firm, fusiform swelling over the right median at the wrist (Figure 3). A biopsy from the skin over the left back was also taken. A piece from the nodule was also sent for mouse foot-pad inoculation on the suspicion that this could be a manifestation of relapse in the nerve by drug-resistant mutants. The patient commenced multidrug therapy.

The contact skin smears from the cut surface of the nodules showed innumerable bacilli, many of them solid. Histopathologically, all three biopsies from the nodular swelling in the nerves showed nerve bundles partly or completely replaced by spindle-shaped cells showing a whorled arrangement (Figures 4 and 5). Another area showed tissue breakdown with many red cells and occasional polymorph. Moderate number of lymphocytes were seen at the

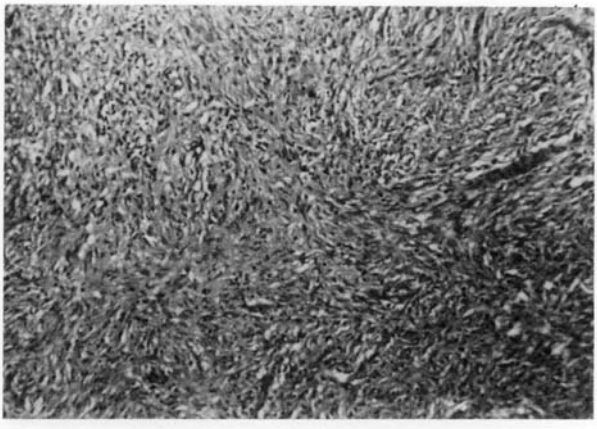

Figure 4. Spindle cells arranged in a whorled pattern, characteristic of histoid leprosy.

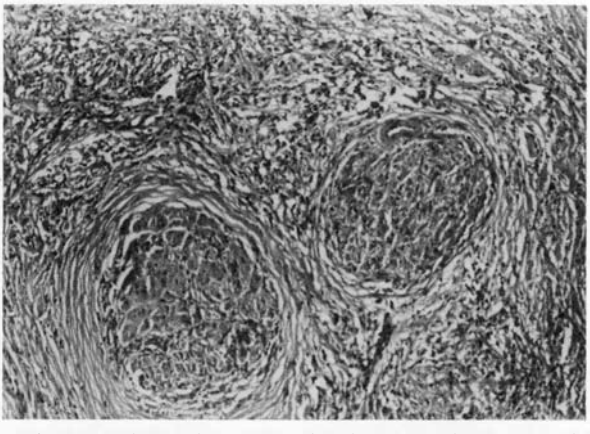

Figure 5. Two fasicles of nerves with perineural lamination and spindle cells with lymphocytes in the adjacent tissue.

periphery of some lesions. Acid-fast stain showed clumps of granular and solid bacilli. The biopsy from the residual skin lesion over the back revealed a picture consistent with resolved leprosy.

Discussion

Histoid lepromatous leprosy manifesting with nodules and plaques in the skin and subcutis have been documented by several workers. The occurrence of such lesions in situations other than those mentioned by Wade have not been recorded except in one instance.[9] Wade, however, observed that 'until detailed case reports

can be presented, little can be said about the clinical variations of the cases presenting with histoid lesions'. Perhaps, he considered that the histoid lesions could occur in other situations as well.

Regarding the genesis of histoid lesions, Wade said that such cases arose in long-standing lepromatous cases who under treatment registered varying grades of improvement, even to the point of negativity in the skin smears, 'but later reactivation or relapse occurred'.[1] As far back as 1963, while on a visit to India, it was demonstrated to him, that histoid lesions can occur, *per se*, in virgin lepromatous cases.[3]

Some workers consider that histoid lesions can arise as an expression of the rapid multiplication of drug-resistant mutants in the multibacillary cases who had been on antileprosy treatment and apparently recovered.[2,4].

It has been postulated[5] that in addition to the general concept that histoid lesions arose as a result of focalized rapid multiplication of drug-resistant *Mycobacterium leprae*, these lesions were essentially an expression of 'an alteration in the growth pattern of the organism in a localized area of skin', and the change in the behaviour of the organism may be due to 'loss of immunity in the localized area of skin', resulting in the enhancement of the bacillary multiplication.

It is stated[6] in an ultrastructural observation of histoid leprosy that 'the histoid lesion is an hyperactive form of lepromatous leprosy, the nature of the stimulus being unknown'. The opinion given is that 'although the histoid lesions are found most commonly in relapsing patients, whether or not the relapse is due to drug resistance, this is not necessarily the case and the stimulus that produces the high cell-turnover has not been elucidated'.

From the foregoing it would appear that the occurrence of histoid lesions could be the outcome of unchecked, progressive lepromatous leprosy or of relapse, the former arising from the unrestricted multiplication of virgin *M. leprae* not exposed to any antileprosy treatment and the latter from drug-resistant bacilli. Apart from these, there is yet another source from where such a phenomenon could occur. These are the nests of 'persisters' which have been identified in no less than five sequestered locations in the multibacillary cases, long after the bacilli had cleared from the skin and the nasal mucosa as determined by standard methods of bacteriological examination. One of the sites of persistence is the Schwann cell. It is said that some of the persisters die in the course of time while others regain normal metabolic activity and multiply.[7] It is not improbable that these persisters in the Schwann cells come out of their hiding and hibernation, become metabolically active, multiply at a prolific rate, producing histoid type of lesions.

It is postulated that the occurrence of firm, non-tender nodular elements in the peripheral and cutaneous nerves in the case under report is a unique instance of relapse occurring exclusively in the nerve, the skin remaining apparently free from relapse lesions; and that the nodules arose as the result of unrestricted

multiplication of drug-resistant bacilli. The results of foot-pad inoculation showed that the organisms were fully resistant to dapsone.

In this context it is of interest to refer to two publications[8,9] reporting on the occurrence of histoid features in the nerve. The presence of histoid features were found in the histological examination of a swelling in the radial cutaneous nerve in a case of histoid lepromatous leprosy, which was mistaken for a nerve abscess. The presence of spindle-shaped cells were observed[9] with many full-stained bacilli in the histological examination of one of the 'large subcutaneous nodules which were attached to the nerve trunks in an untreated lepromatous case'.

This case report on the occurrence of 'neural histoid', though it may now appear to be a clinicopathological curiosity or rarity, it is not unlikely that its frequency can be much more in the present context when drug resistance and microbial persistence continue to be the two serious problems in the therapeutic management of multibacillary leprosy. Perhaps, one may be inclined to pass off these swellings as 'nerve abscess', as almost happened in this case. More cases will be discovered if clinicians are aware of this clinical entity in relapsed as well as active progressive, unchecked lepromatous cases manifesting with histoid features, supported by facilities of histopathological examination.

Acknowledgements

We thank the Department of Epidemiology, SLR & TC, for referring the case, Drs E P Fritschi and S Ramaprasad for exploration and biopsy of the swellings Sri Ramadoss for the clinical photographs, Sri J Ramamurthy for secretarial assistance and patient S for his most willing co-operation.

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Lepr Rev (1984) **55,** 69–76

*SPECIAL ARTICLE—LEPRA PRIZE ESSAY 1982**

Leprosy and the Eye

M T ROGERS

If asked what they knew about leprosy, few doctors would recall how much the eye is affected by this disease. Yet ocular complication is an extremely common finding in leprosy. If left untreated total blindness is the inevitable result which, in the words of ffytche,[11] 'adds immeasurably to the already great burdens that these unfortunate individuals carry because of their peripheral nerve lesions, and frequently the onset of severe visual impairment accelerates the complications of this chronic disabling disease'. This essay, therefore, attempts to produce an overview of 'Leprosy and the Eye', describing the prevalence of ocular complications, the complications themselves and some aspects of their treatment.

'Leprosy is a chronic infectious disease primarily affecting the peripheral nervous system and secondarily involving the skin, the mucosa of mouth and upper respiratory tract, the reticulo-endothelial system, eyes, bones and testes.'[21] It is caused by the bacterium *Mycobacterium leprae* and has been estimated to affect from 12 to 15 or even 18 million people throughout the world. The bacteria have been shown to remain viable from nasal blows for several days but the actual root of infection remains a mystery. Whatever this is, in endemic regions a high proportion of the population come into contact with the bacillus. However, 95% of these mount a normal immune response and do not, therefore, develop leprosy. Of the remaining 5% some are unable to mount any response to the bacteria and so develop lepromatous leprosy. Others develop a hypersensitivity reaction similar to that found in tuberculosis which is, therefore, called tuberculoid leprosy. These are two polar forms of the disease but a full classification of the disease, put forward by Ridley and Jopling,[19] includes intermediate forms of leprosy which demonstrate characteristics of both groups. These are Borderline Lepromatous, Mid-Borderline and Borderline Tuberculoid—though in the Madras classification they are all grouped together as Borderline Leprosy. In addition, sub-polar and neuritic types are described as well as an indeterminate. This last group covers many fresh cases which cannot be classified. Fortunately for many patients this phase may be the only manifestation of the disease and they proceed to healing. But for others it may also, after months, pass into one of the more determinate types of leprosy.

It is evident from this classification that the features of leprosy are very variable according to the type of disease suffered. Moreover, changes in the individual's immune state may result in downgrading or upgrading of the disease and, therefore, add to the variability of the features produced.

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In the early stages of lepromatous leprosy macular, papular or nodular skin lesions are produced. These tend to be hypo- or anaesthetic and may even ulcerate. Macular lesions are generally confined to the trunk, whilst nodular lesions may be found on the limbs and face. As will be seen, spread from these lesions may include the adnexae of the eye or onto the globe itself. Involvement of the nerves does occur but is a relatively late feature of lepromatous leprosy. Depending upon which are involved there may be a sensory or motor dysfunction. Because of the widespread nature of the disease anaesthesia tends to be bilateral with a glove-and-stocking distribution. Unnoticed and repeated painless trauma of the hands and feet, therefore, occurs and ultimately results in ulceration, infection and eventually tissue destruction. Motor nerve involvement, however, produces weakness, wasting and deformity making avoidance and care of trauma all the more difficult. If these were not enough the problems of the untreated patient are compounded further by atrophy and absorption of the small bones of the hands and feet which together with severe eye lesions add cruelly to the suffering of these patients.

In tuberculoid leprosy lesions are confined to nerves and the overlying skin. The nerves are grossly thickened and easily palpable, producing sensory or motor dysfunction as in lepromatous leprosy. However, the lesions tend to be unilateral and are not infrequently confined to only one nerve. Anaesthesia and wasting and paralysis is, therefore, more local and deformity less severe. In addition bone atrophy and primary lesions of the eye do not occur which together with better treatment control make the prognosis for these patients better than that of the lepromatous group.

Borderline patients will have features characteristic of both groups, tending one way or the other according to their level of resistance. As a group, however, neurological symptoms tend to develop earlier and more acutely than in either of the polar forms of the disease.

Most authors would agree that the eye is frequently involved in leprosy. Actual figures, however, vary enormously from 1·5%[1] to 96%[2]. On examination of the papers reasons for these large differences become apparent and relate either to patterns and features of the disease and its distribution or to the protocol of the studies involved. Much of the variation attributable to the disease arises from the fact that ocular complications are more common in the lepromatous form of the disease. Therefore, any variation in the ratio of the lepromatous to tuberculoid patients will be reflected in the overall prevalence of ocular complications. This does, indeed, occur and is itself dependent on both racial and climatic factors such that lepromatous disease is more common in Chinese and Mongolian races and in more temperate climates. In addition, the prevalence of ocular complications is dependent on age, the duration of the disease and the efficiency of its control. Variation of the figures may also be attributed to differences in the protocols of the various studies. Such differences included the population under study (i.e. whether from hospital, the community or leprosaria) and the expertise of and equipment available to the author. For example, an ophthalmologist using more sophisticated equipment such as a slit-lamp microscope in contrast to a leprologist using a simple loupe and torch. Additional variation then arises from what is considered to be an ocular complication, if so, is it of leprous origin and then is it potentially sight-threatening?

Nevertheless, despite these differences and the difficulty of comparison, much information can be gained from these studies, a selection of which will now be discussed.

In a study[3] of 100 patients the high prevalence of facial nerve paralysis was investigated —49%, of which 26% were total and 23% involved the zygomatic branch only. Interestingly 92% of the cases were of lepromatous patients, so clearly facial nerve damage can also be a common complication of this type of the disease. The study suggested that the cold winters of Baba Baghi were directly responsible for the paralysis of the facial nerve, particularly the superficial branches which have 'been conditioned for damage by the invasion and inflammation set up by *M. leprae*'. However, facial nerve damage is normally considered to be secondary to the bacterial invasion itself and not primarily as a result of factors such as the cold. Nevertheless, the high prevalence of this complication at Baba Baghi warrants further investigation. It may be, for example, that the cold

allows greater bacterial proliferation and therefore facilitates the development of this particular complication.

A trial[4] of 466 Nepalese patients from a leprosarium was detailed and included comparisons between age, sex and duration of the disease, as well as the usual comparisons between lepromatous, borderline and tuberculoid patients. He found 90% of lepromatous and 65% of tuberculoid had ocular complications, but included all ocular pathology. Of the 12·7% of eyes which were blind 4·3% could be attributed to leprosy. In addition, ocular complications were more frequent in men and this difference was much more significant in the lepromatous form of the disease where there was a 2:1 ratio. Not surprisingly most ocular complications were more common in the older patients and those with longer standing disease.

Statistics produced in this study[4] are detailed and the differences between the groups quite clear. The actual figures are in fact rather high, but may be accounted for when it is considered that all ocular complications had been included, that 71% of the patients were older than 40 years and that 66% had had the disease for more than 20 years.

Two studies were performed at Port Moresby, Papua New Guinea, just 1 year apart. They are interesting because they were carried out on virtually the same population and, although the aims and protocol of each was slightly different, they produced remarkably similar results, except, that is, when considering potentially sight-threatening lesions of which a prevalence of 12%[5] and 23%[6] were found. These differences could be accounted for because one study[5] only considered lagophthalmos and iritis to be potentially sight-threatening lesions whilst the other[6] included in addition keratitis and bilateral corneal anaesthesia, the prevalence of these accounting for the difference. Using his definition Dethlefs[5] found no potentially sight-threatening lesions in tuberculoid patients and the cases of lagophthalmos were restricted to the borderline group only. Dethlefs compares his overall prevalence of ocular complications, at 52%, with those of another study[7] who found a prevalence of 72%. The difference is attributed to the difference in the mean ages (26 years[5]; 55 years[7]). This suggests that the disease may be detected much earlier at Port Moresby, so enabling early treatment. If this is the case it is, therefore, not surprising that there are no potentially sight-threatening lesions in the tuberculoid patients since their disease is more amenable to treatment and more easily cured. Whilst for the lepromatous and borderline patients involvement of the eye may occur early and is more difficult to treat.

In addition to these findings[5] significant differences for age and the duration of treatment were found[6], and it was noted that the finding of lagophthalmos only in borderline patients agreed with previous findings that significant nerve lesions occur early in this form of disease.

These two studies on essentially the same population of leprosy sufferers are useful, particularly because the younger age of the patients involved enables comparison with other older groups, and shows that on the whole serious eye lesions are less common in the younger population. More interesting still is the finding that in this younger population eye lesions were most common in the borderline group, and that the lesions were all neuritic in origin. Together with a low prevalence of serious ocular complications it is noteworthy that there were no cases of blindness (except three due to trauma). Ree[6] suggests that this may be a reflection of the better care and supervision available at Port Moresby, but also notes that it may merely indicate that no blind patients attend the clinic. If this does reflect the results of better supervision and early treatment it is comforting to note the complete absence of blindness and should make those involved in this field all the more keen to detect these unfortunate individuals early and treat and screen for ocular lesions as soon as and as regularly as possible.

A study[8] was conducted on 430 'mostly fresh' patients attending a leprosy clinic. A prevalence of 25% ocular complications was found which is compared with the 6·3% noted by others[1] who studied 532 patients in Southern Malawi. A somewhat surprising finding was that a high percentage of patients developed eye lesions within 2 years (70% at 1 year: 83% at 2 years) and so it was concluded[8] that 'the duration of the disease seems to play little role in ocular involvement'.

Conversely, a direct relationship between ocular affection and the duration of the disease was found[1]. This apparent contradiction can be understood if it is noted that Sehgal[1] included all ocular complications (and hence also his high prevalence) whilst Ticho & Ben Sira omitted adnexaeal lesions. These lesions tend to occur in the early stages of the disease and, therefore, it is quite probable that their inclusion in Sehgal's[8] figures accounts for the difference. Even excluding this Ticho & Ben Sira's[1] results are surprisingly low, which was considered to be 'due in part to the wide use of sulphones'. If this is the case, then this adds more weight to Ree's[6] conclusion that early treatment reduces the prevalence of ocular complications. In addition, Sehgal[8] considered the possibility of a leprous conjunctivitis since *M. leprae* were found in the smears of 7 out of 8 lepromatous patients with conjunctivitis. Most authors, however, consider conjunctivitis to be due to secondary infection. Since *M. leprae* is found in most bodily secretions, including the tears, of lepromatous patients, their presence in conjunctival smears does not prove a causal relationship with the conjunctivitis of these patients. The possibility of a leprous conjunctivitis should not, however, be dismissed and may form the basis of further study.

A study[9] of 150 patients with ocular complications found a high percentage of males (85%) and the majority of patients over 40 years of age. Binocular involvement occurred in 78% of patients and there was a high prevalence of blindness (65%). This study shows quite clearly the prevalence of ocular complication in the different parts of the eye, demonstrating that affection of the adnexae occurs most commonly. Nevertheless, corneal and uveal tract involvement are also common and more important because they have the potential to cause blindness. Indeed, in a study of blindness in leprosy,[11] it was found that these two complications accounted for 59% of the blind eyes. In Das' study,[9] however, cataracts accounted for 38% of blind eyes, 32% of which were mature senile cataracts and only 6% of which were considered to be secondary to an uveitis.

T J ffytche[11–14] has sought to deal with the problem of cataracts, which although not necessarily caused by the leprosy, can nonetheless have devastating consequences on the often severely disabled patients encountered in his trials. These trials on patients from South Korea produced encouraging results with an improvement of vision in all but three patients (out of 44). He also noted that the mean age of patients requiring surgery was lower than non-leprosy patients suggesting that there may be a stronger relationship between leprosy and the development of cataracts than had previously been supposed.

In a second paper ffytche[13] has considered the pathological processes involved in the uveal changes of leprosy. He produces evidence to support the idea that these are secondary to a neuroparalytic iritis and not due to a primary myositis, as had previously been thought. It was noted that the temperature of the iris is some 3·5°C centigrade below that of the body so making the small unmyelinated autonomic nerves of the iris a prime target for *M. leprae*. He also suggests that just as 'corneal beads' indicate lepromata of the corneal nerves, so 'iris pearls' represent lepromata of the autonomic nerves of the iris. ffytche presents histological, pharmacological and clinical data to support this theory and whilst accepting that there is as yet no absolute proof he believes that the evidence for a neuroparalytic iritis is strong. This, then, would explain the continuing development of ocular complications in those patients who had been treated successfully for many years, since 'the late effects of neuroparalysis include the slow atrophy of the iris muscle and associated chronic low-grade inflammatory changes'.

So far little has been said of how the eye is actually affected. The following account is a résumé of many papers and a few books and deals with the involvement of the eye by structures.

Involvement of the facial (VII cranial) nerve produces some degree of facial paralysis. This may affect the whole nerve or commonly the zygomatic branch alone, producing weakness of the orbicularis oculi muscle and thus partially or totally preventing closure of the eyelids and resulting in lagophthalmos with or without ectropion. Unable to close his eyes the patient is at risk of developing exposure keratitis and secondary corneal scarring. However, many patients are saved from this because of the maintenance of a good Bell's phenomenon, a good tear film and some

residual blink reflex. A few are treated surgically with either a medial or lateral tarsorrhaphy whilst fewer still have been given a 'temporalis transfer'. This enables voluntary closure of the lids by clenching the teeth. Some authors believe that facial nerve involvement is not the only cause of lagophthalmos in leprosy, but that a primary leprous myositis, rigidity of the lids or extensive atrophy of the skin and orbicularis oculi muscle may also be responsible for this complication.

Involvement of the trigeminal (Vth cranial) nerve produces hypaesthesia or anaesthesia of the cornea, which also results in an increased risk of incurring corneal trauma and ulceration with consequent corneal scarring and blindness. The not infrequent involvement of both these nerves can obviously have disastrous sequelae. Direct involvement of either or both is more common in the tuberculoid and borderline forms of the disease. In lepromatous patients involvement is usually a late complication of long-standing disease, but may also result from the acute inflammation accompanying a lepra reaction. Conservative treatment with steroids and atropine may help the latter case.

Adnexaeal involvement is very much more common in lepromatous patients, loss of eyebrows and eyelashes, or madarosis being the most frequent ocular complication recorded. When it occurs the outer third of the eyebrows tends to be affected first and the lower lid before the upper. Hypertrophy and oedema are also common, and nodule formation may produce ectropion and trichiasis, though this complication may be the result of intercurrent trachoma. Epiphora occurs secondary to ectropion, irritation or due to lacrimal duct obstruction. This may occur with primary bacterial invasion or secondary to a dacryocystitis produced by nasal involvement. Nevertheless, despite frequent nasal involvement, florid dacryocystitis is a rare complication.

Conjunctivitis is not an uncommon finding in any form of the disease. It is usually considered to be due to secondary infection which rarely may accompany the aforementioned dacryocystitis but there is also the suggestion that a primary leprous conjunctivitis does occur.

Involvement of the globe itself may also result from direct bacterial invasion of the tissues, but this only occurs in the lepromatous and borderline groups. In the sclera this may result in the formation of lepra nodules or as a more diffuse episcleritis. If severe enough this may develop into a scleritis with secondary atrophy, and staphyloma formation resulting in blindness.

As well as being involved through exposure, the cornea may also be involved by direct spread producing at its simplest corneal nerve opacification and beading. This may progress to pannus or a superficial stromal keratitis and then to an interstitial or even deep keratitis. These last two may result in scarring with or without vascularization and so impede vision, yet the earlier lesions have little affect on vision and usually pass completely unnoticed. Rarely, lepromata may occur in the centre of the cornea with obvious visual defect. This complication has been reported more commonly in South America and Japan, suggesting a regional occurrence. More rarely still a corneal staphyloma may occur which results in blindness.

The uveal tract is frequently involved, but the affects are almost totally confined to its anterior end because of the cooler more suitable environment. A chronic plastic iridocyclitis is the rule but an acute iritis may complicate a lepra reaction or follow the initiation of treatment. Hence previous ocular involvement *per se* is not necessary for the development of this complication. Evidence of such an acute reaction may be found in the presence of flare, cells, a hypopyon or by the presence of keratic precipitates. Secondary glacoma or cataract may complicate the acute attack but most authors agree that these are not features of the chronic form. Indeed, on the contrary, it has been found[15] that the intra-ocular pressure of leprosy patients with chronic plastic iridocyclitis was significantly lower than in other leprosy patients. It is probably a consequence of reduced aqueous production secondary to bacterial invasion of the ciliary body. Eventually this will lead to collapse of the globe and, associated with destruction of the tissue, phthisis bulbi will result. Although most authors do not believe that complicated cataracts follow a chronic iridocyclitis, ffytche suggests[14] that there is a factor which leads to the earlier formation of cataracts and this may be due to the chronic iridocyclitis. Anterior uveitis commonly produces posterior synechiae and iris atrophy.

Some authors believe this to be a primary myositis; but some others[12–14,16] believe this to be secondary to neurotrophic changes, as has already been mentioned. Patchy atrophy may result in an acquired coloboma of the iris which is accentuated by the irregularity produced by the posterior synechiae. If severe enough it may result in the formation of 'many pupils', or polycoria. Accompanying the atrophy, debris and exudates are formed, which may collect around the pupil margin as floccules or, if sufficient, may result in total occlusion, 'occlusio pupillae' and blindness. Miosis is also a feature of chronic iridocyclitis, initially resulting from autonomic nerve involvement and later from the consequent preferential atrophy of the dilator muscle. Together with the formation of posterior synechiae this may result in seclusion of the pupil, 'seclusio pupillae', also producing blindness.

Cataracts are a common cause of blindness in leprosy patients. The majority are simple mature senile cataracts but as has been discussed complicated cataracts may follow an acute iritis. Whether or not they complicate the chronic plastic iridocyclitis of leprosy, however, still has to be evaluated.

Involvement of structures posterior to the lens is more debatable though a posterior uveitis has been reported, as well as nodular and macular lesions. Even so these are usually confined to the periphery of the eye and represent extensive ocular involvement.

The best treatment for most of these complications is the removal of the cause, *M. leprae*. In the past this was not possible but the advent of the sulphones in the form of promin (1943), and then dapsone, produced some hope. For a long while dapsone has been used alone but in more recent years dapsone resistance has become a major problem. This, therefore, formed the major part of the discussions of the WHO study group on 'Chemotherapy of leprosy for control programmes'.[17] As a result the study group recommended that dapsone monotherapy be abolished and its use with only one other drug is not advised because of the risk of developing resistance to both drugs if dapsone-resistance *M. leprae* are present. For this reason multidrug regimens have been recommended and include the use of rifampicin with dapsone and clofazimine or if the latter is not acceptable, because of the development of red/blue skin pigmentation, then either prothionamide or ethionamide are acceptable alternatives. This regimen is suggested for multibacillary cases because of the greater likelihood of developing resistance. However, for paucibacillary cases where this risk is minimal rifampicin therapy alone should suffice. However, the addition of dapsone has been recommended by the study group in order to avoid the emergence of rifampicin resistance in those who have been wrongly diagnosed as paucibacillary. Indeed, such resistance has already been described[18] in two lepromatous patients who were treated with rifampicin monotherapy.

Specific treatment for eye complications includes plastic surgery for madarosis or trichiasis, or in the latter case epilation or electrolysis of the hairs may be effective. Reduced tear secretion may be treated medically with artificial tears, whilst problems with drainage are better treated surgically to establish a new drainage system. If the cause is chronic dacryocystitis, a dacryocystectomy is indicated. Acute and sub-acute infections are better treated with antibiotics and irrigation of the lacrimal sac. Paralysis of the ocular muscles may also be treated with artifical tears or some protective device, such as glasses or goggles. Active exercises and education into voluntary blinking for those with some residual function has been suggested, whilst tarsorrhaphy may be a more lasting solution for those who have not. On the whole infiltrative disease is best treated with anti-leprosy therapy, though individual lesions may require excision. Finally, an acute inflammatory reaction may occur in either the infiltrated eye or as a consequence of circulating degradation products following treatment. In the past therapy was immediately stopped but now it is advocated that anti-leprous therapy should be continued. If mild, simple analgesics and a warm compress may be sufficient to reverse the inflammation and relieve the pain. If more severe, then mydriatic and cycloplegic agents will be required to prevent the production of synechiae whilst the inflammation is treated with NSAID or steroids. If the eye alone is involved drops or ointments can be used but more generalized effects should be treated with systemic steroids. Secondary glaucoma may rarely occur and this is best treated in the conventional manner with acetazolamide whilst anti-inflamma-

tory medication is continued. If severe enough, however, this too may require surgery in the form of an emergency iridectomy.

What then has so far been accomplished?

The actual ocular complications have now been widely studied and certain features attributed to the different forms of leprosy. Allied to this it is now recognized that there is a marked regional variation in these ocular complications according to race, climate, type of disease, sex, age and duration of the disease. Certain studies have demonstrated that early effective treatment of the disease as a whole can prevent the development of ocular complications, whilst others have shown encouraging results in the management of symptoms or the treatment of intercurrent disease such as cataract. More also is known about the pathology of ocular involvement so that many of the complications can now be explained rather than simply witnessed and documented. In this respect the significance of the chronic plastic iridocyclitis seen in so many lepromatous patients is now better understood and the importance of early treatment to prevent further neural damage better appreciated. Finally, in the words of ffytche:[11]

> Visual problems in patients with leprosy very often have an effect that far outweighs their severity in normal individuals. The life of the leprosy patient is of necessity one of constant care and attention to the anaesthetic areas of the body in order to avoid trauma and infection; and visual surveillance of the extremities becomes a code to be practised daily by sufferers of this disease. Even a minor abrasion or wound may lead to eventual loss of a digit or permanent impairment of the function of a limb. Blindness if it occurs for any reason adds immeasurably to the already great burdens that these unfortunate individuals carry because of their peripheral nerve lesions, and frequently the onset of visual impairment accelerates the complications of this chronic disabling disease.

And then as Reddy[20] concludes:

> In the early stages the involvement of eye tissues may not produce any symptoms or inconvenience to the patient. However, if they are left untreated they may lead to serious complications. It is therefore essential that in all patients suffering from leprosy, the eyes should be examined carefully and periodically even though the patient does not complain of any eye symptoms. By doing so, the eye complications can be detected in their early stages and their timely treatment will prevent the serious complications and blindness.

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SPECIAL ARTICLE

Acworth Leprosy Hospital, Bombay

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Harry Acworth, a principal founder of the Acworth Leprosy Hospital, joined the Indian Civil Service in 1870 aged 21. He was educated at Brighton College and Oxford and qualified as a barrister before going to India, where he served 25 years in the Bombay Presidency (Maharashtra and Gujarat), ending his career, 1890–95, as Municipal Commissioner of Bombay. On retirement to Malvern, he got to know and to play golf with Elgar and he was also active in local government and church affairs until deafness cut him off more and more from the world. My clearest memory of him during the last years is the clatter of his machine as he typed Braille books for the blind.

The above is a brief sketch of the man whose name still stands over the gateway of the Acworth Leprosy Hospital in Bombay, which he did so much to establish.

In June 1890, a Lady Thompson wrote to the *Times of India* drawing attention to the desperate state of lepers on the streets of Bombay and pleaded for a home to be built for them. This led to a public meeting at which the idea was not too well received and a mere Rs 12,000 were raised. It was then that Acworth took matters in hand, risking, so the *Times* thought, his reputation as the new Municipal Commissioner (with the distinct chance of a fiasco).

He undertook 'immense' labours himself, personally visiting and collecting donations from shopkeepers until, in February 1891, he was able to report to a 'jubilant' meeting his 'triumph' in raising Rs 73,000. By then he had already established 200 leprosy patients in the 'model' Matunga Asylum, which was officially opened on 7 November 1890.

This was the first time in India that the principle of compulsory restraint was applied to the victims of leprosy. The laws, first of Bengal and then of the Government of India itself, were based on the experience of Matunga. The reduction of vagrancy and of infection on the streets was coupled with a benign project of genuine welfare benefit. That the compulsory element was not onerous is shown by the fact that volunteers came from far and wide to the Asylum. Indeed, some cottages were soon built for paying patients. Running costs were covered jointly by the Government and the City Corporation at a monthly rate of Rs 10 per patient.

The title of the institution changed as it grew and as its functions developed: 1904, Acworth Leper Asylum with 351 beds; 1935, Acworth Leprosy Home with 399 beds; 1956, Acworth Leprosy Hospital with 500 beds.

In 1911 a Board of Management of 20 members under the presidency of the Municipal Commissioner was established under the Leprosy Act of 1898, but it was not until 1929 that the Board appointed a Medical Advisory Committee. However, the Asylum still remained just that, providing domiciliary in-patient treatment and sheltered workshops for its few hundred inmates.

At last, in 1935, out-patient treatment started, and in 1939 the Superintendent began training

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courses for the staff of Government and Municipal Dispensaries, thus extending more widely the possibilities of early detection and treatment. This led, in 1942, to the appointment of the first Health Visitors to follow-up patients and their contacts.

Over the years the buildings had been constantly extended to accommodate more patients and to expand the facilities. By 1939 there was a laboratory and lecture hall, and in 1950 an operating theatre was built. In 1963 a Craft Training Centre and Sheltered Workshop were provided by the Tata Trusts and some 10 years later the physiotherapy block was opened.

Acworth first used promine for treatment in 1946 and dapsone 5 years later. The latter drug was first tried out there in 1956 for prophylactic treatment by para-medical workers.

Meanwhile, thanks to the efforts of Dr Wardekar, in 1955 the Greater Bombay Leprosy Control Scheme (GBLC) was started with 5 clinics for Survey, Education and Treatment (SET). This number doubled in the next 15 years. The Superintendent of Acworth supervised the medical work of the Scheme and in 1959 its management was transferred from the Corporation to the Board of the Acworth Leprosy Hospital.

From 1950 to 1961 the Indian Council of Medical Research had conducted an inquiry into leprosy there. Subsequently, the hospital set up its own Research Unit. It is surprising to note that it was not until 1956 that leprosy training was included in the MBBS degree course as well as in the Ayurvedic Faculty. These were both undertaken by Acworth.

In 1964 and 1965, the Hospital received an award for the voluntary institution doing the best leprosy control work.

My wife and I were able to visit the Hospital several times in the course of our travels for Oxfam, most notably in 1969 for the celebration of Gandhi's centenary. In company with the Municipal Commissioner, we unveiled and garlanded the bust of Harry Acworth, for which his friends had subscribed 79 years before, and we planted commemorative shrubs.

It may give some idea of the scale of operations to record that in 1969 Acworth and the GBLC Scheme had then, annually: a turnover of Rs 10 lakhs; 91 staff; 15,800 old patients; 5700 new patients; 5500 social workers' interviews; 60 lectures and seminars; 12 exhibitions; 337 physiotherapy patients; 29 plastic and reconstructuve surgical operations; 7800 house visits; 7 research projects; 682 in-patients and training in and production of carpentry, weaving, tailoring, leather and vegetable production. They told us they had 60,000 patients on their books.

A postcript. In 1898 Harry Acworth read a paper on 'Leprosy in India' at the Imperial Institute, London. Dr Armauer Hansen characterized it as the ablest statement of the case which he had ever heard from a non-medical man.

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Obituaries

DR GORDON CURRIE, OBE, MB, CHB, FRCGP, DTM & H 1922–1983

Gordon Currie was born to a missionary family serving in Malawi (Nyasaland). His father retired to Glasgow in 1932 where Gordon attended school and studied medicine at Glasgow University, qualifying in 1944.

In 1946 he was accepted for service with the Church of Scotland Missionary Society and offered the opportunity of working with leprosy patients at Itu in Eastern Nigeria. This he declined and was posted instead to the Livingstonia Mission in the North of Malaŵi to train para-medical staff. It was there that he first came into contact with the problems of leprosy treatment as his duties included the supervision of a small leprosy settlement at Bandawe on the shore of Lake Malaŵi.

At about this time the use of DDS in the treatment of leprosy was being re-examined. Gordon Currie was quick to see the possibilities of this oral therapy and, on being approached by the Director of Medical Services, joined the government service and was appointed to take charge of a new Government Leprosarium which was under construction in the Central Region. This institution was later to be named 'Kochirira'—'place of healing'.

In preparation for his duties there he spent a month in Zimbabwe (Southern Rhodesia) and 5 months in Kenya, studying leprosy treatment methods in those areas. In 1955 he was granted a WHO fellowship to work with Dr T F Davey in Nigeria, where the out-patient treatment of leprosy was already well established.

On returning to Malaŵi, he set about establishing treatment for leprosy patients at the numerous Government and Mission rural dispensaries throughout the country, visiting every medical unit at least once a year to lecture, demonstrate and encourage.

He was awarded the Malaŵi Independence Medal in 1964 and appointed Leprosy Specialist by the Ministry of Health. In this capacity—and using his wide knowledge of the country, enhanced by an aerial survey of likely areas—he was closely involved with the setting up by LEPRA of the Pilot Leprosy Control Project in 1965 and in selecting suitable para-medical staff for the mobile diagnosis and treatment units which subsequently proved to be highly successful in reducing the incidence of the disease.

In 1966 he was awarded the OBE in recognition of the services he had rendered to the health of the country.

While he was always primarily occupied with the practical day-to-day treatment of leprosy patients, he still was keenly interested in, and contributed to, the clinical trials of new drugs or application methods.

His published papers included: 'Clinical trial of CIBA 16095E', in collaboration with Dr T F Davey (1956); 'Short and long acting sulphones by intramuscular injection', (1959); 'A clinical trial of etisul in lepromatous leprosy', (1963); 'A clinical trial of sulforthomidine in lepromatous leprosy', (1966).

It was a great loss to the leprosy control programme in Malaŵi when family affairs made it necessary for him to retire from the service and return to Scotland in 1966.

In 1967 he joined a general practice in Glasgow and very quickly directed his tremendous store

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of energy into this different branch of medicine, quickly becoming a leading GP in the city. He was elected to numerous medical committees and yet still found the time to study for, and pass, the examination of the Royal College of General Practitioners, and was elected Fellow in 1981.

He became hospital practitioner at a Geriatric Unit in the city and planned the 'Medical and social screening of patients aged 70 to 72 by the health team of an urban general practice' (*Br Med J* 1974). He contributed chapters on 'The Care of the Elderly', 'The Care of Terminal Illness' and 'Geriatric Care' for the textbook of *General Practice Medicine*.

A kindly, understanding and courteous man, he could be fierce when dealing with indifference and ingrained prejudice.

For 2 years before his death, Dr Currie knew that his time was limited, but did not allow this to affect his work, and only close members of his family were aware of his condition.

Dr Currie was not a man who sought publicity or public acclaim but, in addition to the members of his family, an indication of the high esteem in which he was held was shown by the large number of people, from all facets of his life, who attended the funeral.

The Rev. Angus Turner, in his tribute at the funeral on 15 September 1983, said '. . . he was first to last an explorer, always pushing out the frontiers of his experience professionally and within his faith . . . he was impatient with moribund orthodoxy wherever he met it . . .'.

Dr Currie leaves a widow and son in Glasgow, and a married daughter in the United States.

DOUGLAS COFFIN

JOSE G TOLENTINO, MD 1900–1983

It is with deep regret that the Leonard Wood Memorial announces the death of Dr Jose G. Tolentino. Before his retirement in 1971, Dr Tolentino had been Chief of the Clinical Research branch of the Leonard Wood Memorial Laboratory for Leprosy Research in Cebu, Philippines. He had held this post and that of Research Leprologist since 1951. Prior to that time, Dr Tolentino had been resident Physician of Southern Islands Hospital, Senior Physician for Culion Leper Colony, Resident Physician of the Eversley Childs Sanitarium, Chief of the Eversley Childs Sanitarium, Physician-In-Charge of the Cebu Skin Clinic and Assistant Director of the Cebu General Hospital.

Dr Tolentino received his MD degree from the University of the Philippines in 1926 and dedicated his professional career to the leprosy cause. He was considered to be among the most outstanding clinical researchers in the field and since 1952 was influential at numerous conferences dedicated to this important work.

EXECUTIVE DIRECTOR

Leonard Wood Memorial
(American Leprosy Foundation)
11600 Nebel Street
Rockville, Maryland 20852, USA

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Domiciliary and Field Work

The Implementation of Multi-drug Therapy; Staff Training Manual. **The Leprosy Mission (International), London, 1983.**

This is a paperback manual of 20 pages, written by Dr (Mrs) E S Thangaraj and Miss Jane Neville. The introduction stresses the great importance of ensuring that medical personnel are properly trained in order to ensure the safe and effective use of multidrug therapy on a mass scale. This training is considered in relation to medical officers; non-medical supervisors; paramedical workers; health educators; nurses and laboratory technicians. In each case, tasks and learning objectives are listed in detail. Taken together with the system of distribution of appropriate health learning materials for leprosy which has already been developed so well by the Leprosy Mission (International) London, this manual should be of the greatest value, not only in India (where it was published), but also in many other countries where multidrug therapy regimens will be used.

Teaching and learning materials in leprosy. The Leprosy Mission (International) London

We have received the most recent issue of the materials which are available from this centre and they are as follows:

1 **A Guide to Leprosy Control:** *WHO Geneva 1980*—A statement of WHO principles and policies to assist those who plan, fund and operate leprosy control programmes.
2 **WHO Expert Committee on Leprosy:** *WHO Geneva 1977*—5th Report WHO Technical Report Series No. 607.
3 **Chemotherapy of Leprosy for Control Programmes:** *WHO Geneva 1982*—Report of a WHO Study Group. Technical Report Series No. 675. Contains WHO recommendations for multidrug regimens for use in leprosy control programmes.
4 **Guidelines for the Campaign Against Leprosy:** *ILEP (International Federation of Anti-Leprosy Associations) 2nd Edition 1982*—This booklet sets out principles for the conduct of leprosy control programmes.
5 **Memorandum on Leprosy Control:** *S G Browne. Oxfam, Lepra and The Leprosy Mission 1980*—Outlines in simple and non-technical language the modern approach to leprosy control.
6 **Diagnosis and Management of Early Leprosy:** *S G Browne. The Leprosy Mission, London 1983*—Small illustrated booklet.
7 **Leprosy:** *A Bryceson and R E Pfalzgraff. Churchill Livingstone 1979, 2nd Edition*—Covers principles involved in the study of leprosy as well as the clinical and social aspects of the care of leprosy patients.
8 **Handbook of Leprosy:** *W H Jopling. Heinemann Medical Books Ltd. 1978, 2nd Edition*—Deals with diagnosis, immunological concepts, classification, treatment and management of leprosy. Excellent glossary.
9 **Essentials of Leprosy:** *J M H Pearson and H W Wheate. ALERT, Addis Ababa 1980, 4th Edition*—A good review of the epidemiology, clinical features, diagnosis, complications and treatment, rehabilitation and control of leprosy.
10 **A Manual of Leprosy:** Editor: *R H Thangaraj, New Delhi, India 1983, 3rd Edition*—First half deals with diagnosis, classification and treatment of leprosy. Eight chapters cover prevention of deformity, P.T., O.T., social aspects and surgery. Two chapters on leprosy control.
11 **Leprosy in Children:** *F M Noussitou. WHO Geneva 1976*—Excellent colour photos.
12 **A Guide to Leprosy for Field Staff:** *ALERT, Addis Ababa, 1977*—Written for paramedical workers who care for leprosy patients in outpatient clinics. Could be translated or adapted for local training of health staff.
13 **A Practical Guide to the Diagnosis and Treatment of Leprosy in the Basic Health Unit:** *H W Wheate and J M H Pearson. ALERT 1979*—written to help general health staff diagnose leprosy early and to initiate treatment. Written simply, could be translated.
14 **Drug Resistance in Leprosy:** *S G Browne. The Leprosy Mission, London, 1983*—pamphlet.
15 **Physical Therapy in Leprosy for Paramedicals:** *E D Kelly. American Leprosy Missions Inc., 1981*—This training manual is in 3 parts—Level 1 teaches middle level paramedicals about care of insensitive hands, feet, eyes. Level 2—more anatomy and further exercises. Level 3—for training techniques for pre and post operative care in reconstructive surgery unit. Levels 1 & 2. Level 3.
16 **A Guide to Health Education in Leprosy:** *P J Neville. ALERT 1980*—Booklet offers topics and information for leprosy patient education. Suitable for translation and adaptation to local situations.
17 **Better Care in Leprosy:** *M Laugesen*—Pocket-sized booklet to teach rural health workers in India about leprosy. Ideas and format could be adapted for other countries, and text translated.
18 **A Simple Sandal:** *P J Neville, ALERT, 1981*—Two chapters extracted from larger Manual on Footwear. The booklet contains information for the construction of simple footwear, which should be adapted to local conditions.
19 **Insensitive Feet:** *P W Brand, The Leprosy Mission, London 1981*—A practical handbook on foot problems in leprosy.
20 **A Footwear Manual for Leprosy Control Programmes:** *Editor: P J Neville.*—Part I contains information for the administrative and medical staff of footwear programmes. Part II contains construction details for the technician. (Two volumes)

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21 **The Care of the Eye:** *Margaret B Brand. The Star, Carville, USA*—Deals with the recognition and management of eye complications in leprosy.
22 **The Social Dimension of Leprosy:** *ILEP 1982*—A training manual for health workers, based on the case study method.
23 **Skin Biopsy in Leprosy:** *D S Ridley. Ciba-Geigy 1977*—For research and laboratory workers and clinicians who make use of biopsies in leprosy.
24 **Partners:** *The Leprosy Mission, London*—Magazine for paramedical workers in leprosy. Two issues a year.

In order to help you select appropriate material a check list is provided below. Numbers refer to titles on the booklist.

Check list for:	**Recommended books:**
Doctors, Supervisors	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 19, 20 (Part I) 21, 22.
Senior Health Workers, Nurses	3, 5, 6, 7, 8, 9, 10, 11, 14, 15, 19, 22, 24.
Health Educators	5, 10, 14, 16, 17, 19, 22, 24.
Shoe Workshop Managers	19, 20 (Parts I and II).
Shoemakers	16, 18, 20 (Part II), 24.
Junior Health Workers	12, 13, 14, 16, 17, 24.
Specialists	21, 23.
Medical Students	7, 8, 9, 10.
Health Programme Planners	1, 2, 3, 4, 5, 20 (Part I).
Laboratory Technicians	10, 14, 24.
Physiotherapy Technicians	10, 15, 16, 24.

ILEP Catalogue on Training, 1984

This excellent catalogue from ILEP (The International Federation of Anti-leprosy Associations, London), lists eight main centres where training in leprosy is carried out and gives details of their 1984 programmes. With some modifications, it can be expected that similar programmes will continue in future years, as they have in the past. The centres listed are—ALERT in Addis Ababa, Ethiopia; The Institut Marchoux in Bamako, Mali; The Hospital 'Lauro de Souza Lima' in Bauru, Brazil; The National Hansens's Disease Center in Carville, Louisiana, USA; The Sanatorio de Fontilles in Alicante, Spain ; The Schieffelin Leprosy Research and Training Centre in Karigiri, India; The Centro Dermatologico Pascua in Mexico City, Mexico; The Centre d'Enseignement de l'OCEAC in Yaounde, Cameroun.

The introduction of combined chemotherapy regimes for leprosy. ILEP, 1983

This booklet, which has been prepared by the ILEP Medical Commission, is now ready for printing. It will appear in A5 format, 30 pages, in a bilingual French/English version, as an ILEP publication.

Its purpose is to assist ILEP Member-Associations in their plans to introduce effective multiple drug chemotherapy in the programmes which they support, and to give practical guidance which they can transmit to the staff supervising these programmes.

Recognizing the widely different conditions in which leprosy programmes operate, the booklet covers broad principles on which policies and practice can be based and does not go into details. It is intended to meet the immediate need to introduce MDT as rapidly and as widely as possible. Member-Associations are asked to take into account the fact that, as practical experience is built up, the booklet will be revised and updated until it is possible to make longer-term recommendations.

Members of ILEP are being asked to submit their requests for copies of this booklet to the Coordinating Bureau, 234 Blythe Road, London W14 0HJ

The Control of Tuberculosis and other publications by AKAP (Philippines) and World Neighbors (USA)

Margaret Blake, Secretary for Development Communications in World Neighbors, 5116 North Portland, Oklahoma City, Oklahoma 73112, USA has kindly written to say that:

'World Neighbors is an organization which assists development programs in many countries throughout the world. Supported entirely by private funding, we work with other international organizations, as well as local groups, to help persons in rural areas of developing countries improve their lives by utilizing their skills, talents and resources. Our cooperative work takes the form of monetary assistance, technical know-how, and perhaps most important, shoulder-to-shoulder help toward stated goals. When the local program personnel can carry on the work alone, World Neighbors moves on to another area or another program.'

This organization produces a range of materials for person-to-person education in health and community development etc., and these include filmstrips, flipcharts, books, booklets and pamphlets, together with two newsletters. The film strip on the control of tuberculosis has 46 colour transparencies with a two-page brochure which reproduces the pictures in black and white and carries

an explanatory text for each one. There is also an excellent flip-chart of 32 pages on the control of tuberculosis, produced jointly by World Neighbors and AKAP in the Philippines (66 J P Rizal, Project 4, Quezon City). These community-based materials are thoughtfully and effectively produced and---despite what is already available—might well be adapted for use in leprosy.

OXFAM–LEPRA pack of 25 documents for teaching and learning in leprosy

Following discussions in late 1981, 50 packs were assembled with the objective of providing an 'immediate' source of information for medical students, doctors, programme planners and supervisors, nurses, tutors—and other potential teachers. A further 50 were assembled in 1982 and this first 100 sold out in 7 months. A further 100 were produced in order to cope with continuing requests and now (September 1983) only a few remain. Discussions are in progress on the content of a 'mini-pack' which will give greater emphasis to multiple drug therapy.

Histopathology Services for Developing Countries

Professor Michael Hutt retired from St Thomas's Hospital in London in September 1983 and recently issued the following letter concerning histopathology services, which include the examination of biopsies for leprosy.

'For the last 15 years the department of Histopathology at St Thomas's Hospital has provided a free, postal, diagnostic service for a number of hospitals, both government and mission, in developing countries. It was originally envisaged that the need for such services would decrease as they were built up locally. For a variety of reasons, differing from country to country, this has not happened and the need is still there and likely to continue. To meet these problems and to provide histopathological expertise in parasitic, communicable and other tropical diseases in the UK, a new consultative histopathologist post has been created jointly with London School of Hygiene and Tropical Medicine and University College Hospital Medical School. This post has been filled by the appointment of Dr S B Lucas who has spent 2 of the last 4 years in this unit and 2 in the Pathology Department at Nairobi. My own full-time post will terminate in September when I retire, though I will continue my involvement with developing countries on a part-time basis.

Dr Lucas is keen to maintain or increase the diagnostic services for tropical countries and we hope to raise funds to cover the expenses of such work.

'As from 6 April 1983, I would be grateful if you could re-route your postal specimens to him:

Dr S B Lucas, Department of Morbid Anatomy, School of Medicine, University College London, University Street, London WC1. Telephone 01-387 9300.

'I hope to remain in contact with you through my association with Dr Lucas and I am sure that he will provide you with an excellent service.'

M S R HUTT, *Professor of Geographical Pathology*

LEPRA Prize Essay Competition for Medical Students in the UK 1984

Following the tradition of the past 10 years or more, LEPRA is organizing a prize essay competition for UK medical students in 1984, with the following titles—'Monoclonal antibodies and recombinant DNA technology; present and future use in leprosy and tuberculosis' OR 'Leprosy will be most expediently controlled by the continued use of vertical, specialized programmes' OR 'Leprosy will be most expediently controlled by the use of fully integrated programmes which make use of the primary health care approach'. Full details will be sent to all medical schools in the UK early in 1984. Enquiries may also be directed to the Editor of this Journal, or to LEPRA, Fairfax House, Causton Road, Colchester CO1 1PU.

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Reports, News and Notes

LEPRA; 60th Anniversary Year, 1924–1984

LEPRA celebrates its Diamond Jubilee in 1984. The Association, initially known as the British Empire Leprosy Relief Association (BELRA), was set up in 1924 by a former secretary to the Mission for Lepers in India, Frank Oldrieve, Sir Leonard Rogers an ex-Major General in the Indian Medical Service, and philanthropist Sir Frank Carter. With the Prince of Wales as its patron and the support of a wide range of notable and interested people, the Association was launched with the aim of ridding the British Empire of leprosy. Highlights of the Diamond Jubilee year will include—a £100,000 jewellery appeal: 'An Eye for a Gem, a Gem for an Eye', to be launched in January, the proceeds to be used to prevent blindness in leprosy sufferers; a prestige auction of the jewellery donated to this appeal; an anniversary thanksgiving service in All Hallows by the Tower, London in February; major UK participation in the 12th International Leprosy Congress in New Delhi this year, and a wide range of fund-raising and publicity events by LEPRA branches throughout Britain in 1984.

Simple Orthopaedic Aids; appropriate designs for a developing country: Chris Dartnell

This is a paperback of 45 pages, approximately A4 format. The introduction reads:

'The book aims to set out in simple detail how to set up a workshop and produce these orthopaedic aids in a country where resources are scarce but the need is very great. The Khartoum Cheshire Home is taken as the model but the principle involved is applicable, with slight modifications, to meet most other situations. The information is intended for physiotherapists, nurses, health workers, heads of Projects; local workers; in fact anyone wanting to produce aids for the rehabilitation of handicapped people.'

A press release adds the following information:
European aids for the handicapped are not suitable for Third World countries where the terrain varies widely, local labour is not expert and sophisticated materials are too expensive or not available. Yet though resources are so limited, the need for aids is very great and the provision of them can mean restoring a man or woman from helplessness to leading a useful, meaningful life. *Simple Orthopaedic Aids* covers how to set up a workshop with full details and simple drawings on the production of orthopaedic aids for the rehabilitation of the handicapped using locally available materials and technology appropriate to the circumstances. These aids include a wide range of callipers, clogs, shoes, walking frames, a basic pylon, splints and wheelchairs. It will be an invaluable guide to anyone working in or near to the field of rehabilitation for handicapped people in underdeveloped countries.

Available from Chris Dartnell, The Leonard Cheshire Foundation International, Leonard Cheshire House, 26–29 Maunsell Street, London SW1 2QN. Price £2.50 plus 30p postage and packing per copy. Overseas, £2·00 per copy postage and packing.

An Atlas of Leprosy: Sasakawa Memorial Health Foundation, 1983

We acknowledge with thanks receipt of this beautifully produced atlas from the executive and Medical Director of SMHF, Tokyo. This is a strongly constructed, loose-leaf paperback of 57 pages and represents a revised version of the 'Atlas' which was issued by SMHF about 2 years ago. The first 42 pages have large colour pictures of virtually all aspects of clinical leprosy; pages 43–51 illustrate the main histopathological findings; and the remainder of the 'Atlas' is devoted to differential diagnosis. The quality throughout is of an exceptionally high standard and this is without doubt the best atlas of its kind so far produced for the study of leprosy. Many of the photographs, especially those of children, approach life size, and would be invaluable as an exhibit in a teaching centre and also easily visible at a range of several metres during small-group teaching. Dr Yo Yuasa indicates in his Postscript that 3000 copies have been produced and that it is hoped to '. . . distribute this edition to many stations in the field where peripheral health workers are expected to recognise or even diagnose and classify various types of leprosy, in addition to training centres and educational institutions.'

The Foundation is to be congratulated on the production of this Atlas, which should be of the greatest value to all who are concerned with clinical and histopathological aspects of leprosy.

Reconstructive surgery in leprosy: 1 Opponens plasty; 2 Correction of clawed fingers—colour transparency sets and text. N H Antia and S G Kamat, Bombay, 1983

These excellent slide sets describe two of the most important operations for deformities of the hand in leprosy. The first has 24, and the second 48 colour slides of high quality, which are designed '. . . to provide an easy introduction to the subject and stimulate interest for further reading and for undertaking surgery.' The text is extremely clear for both operations and could be used either for self-instruction or teaching others. The cost of (1) is Rs.250 and of (2) Rs.500. Enquiries to Dr N H Antia, Ben Nevis, Bhalabhai Desai Road, Bombay-400 036, India.

Symposium on monoclonal antibodies in Reading, UK, April 1983

Ortho Diagnostic Systems sponsored a 1-day symposium, attended by 250 participants in April 1983, which was aimed at the presentation of an overview of the potential of lymphocyte markers in helping with the diagnosis of a wide range of disorders. It was commented that the use of OKT reagents can both clarify diagnosis and monitor response to therapy. All speakers produced data to show that the ratio of T-helper cells (OKT-4 positive) to T-suppressor cells (OKT-8 positive) was changed in disease states or in response to therapy. The subject was dealt with by four main speakers under the headings of: Monoclonal antibody production and application; malignant melanoma monitoring; T-cell detection and thrombocytopenia; T- and B- cell involvement in bone marrow transplantation and monoclonal antibodies and instrumentation. Source: *Medical Laboratory World*, June 1983, 33 Bowling Green Lane, London EC1B 1EH.

Heiser Research Progam for Research in Leprosy, 1984

This well-known organization has issued details of the awards which will be available in 1984. As in previous years, these are offered under the headings of: postdoctoral research fellowships; research grants; visiting research awards. There is an excellent summary of the state of 'Leprosy Research Today', which gives some indication of the priorities this organization has in mind. The Scientific Advisory Committee is composed of Maclyn McCarty, Lane Barksdale, Barry Bloom and Charles Shepard. Further details from Mrs Barbara M Hugnet, Director, Heiser Program for Research in Leprosy, 450 East 63rd Street, New York, New York 10021, USA.

Raoul Follereau Grant for Leprosy Research, 1984

The Italian Leprosy Relief Association 'Amici di Raoul Follereau,' an organization for international health cooperation, offers a grant of US $20,000 for leprosy research, named after Raoul Follereau, to a young research worker in a European department. The object of the grant is to stimulate the undertaking of original research in the field of leprosy in a research department in Europe.

The precise conditions were finalized at the recent meeting of the Federation of Anti-Leprosy Associations (ILEP) in Berne, Switzerland in June 1983, and will shortly be distributed to all ILEP members, leprosy journals and appropriate institutions, including universities and research centres, in Europe. Meanwhile, further details may be obtained from 'Amici di Raoul Follereau,' via Borselli, 4—40135 Bologna, Italy. Tel. 051/423809—433402.

Damien–Dutton Award for Dr Ma Haide, Peking, China

Dr Ma Haide (George Hatem), a well-known Chinese dermatologist, was honoured with the 1982 award for Leprosy Aid by the Damien-Dutton Society on 16 April at the United States embassy in Beijing. The society, through its president, Howard E Crouch, asked the American Ambassador Arthur W Hummel Jr to officiate at the ceremony.

The American-born doctor first came to China in 1933 and joined the Red Army in North China in 1936 to do wartime medical work. After 1949, he took part in the drive to wipe out venereal disease and leprosy. After the former was eliminated in the late 1950s, he concentrated on leprosy. Invited by the Damien-Dutton Society, Dr Ma and two other Chinese specialists made a medical tour of eight countries in 1982.

Speaking at the ceremony, Dr Ma said that in 1949, China had an estimated half a million leprosy patients. Now the number has dropped to about 200,000. He pointed out that China is aiming at the full control and basic eradication of leprosy by the year 2000. The country has 10,000 full-time medical personnel, 86,000 beds and more than 1100 institutions, hospitals and villages to fight leprosy. The cost of treatment is paid by the state. A basic food subsidy is provided for all institutionalized patients.

The award was inaugurated in 1953 and named after the Belgian priest Father Damien de Veuster and the American layman coworker, William Dutton. Source: *Chinese Med J*, 1983, **96:** No. 7.

Technical Guide for Smear Examination for Leprosy by Direct Microscopy

Published by the Leprosy Documentation Service (INFOLEP) at the Royal Tropical Institute, Mauritskade 61a, 1092 AD Amsterdam, the Netherlands, this 34-page paperback booklet covers all main aspects of smear examination. It was produced with the support of the Netherlands Leprosy Relief Association and the Ordre Militaire et Hospitalier de Saint Lazare de Jerusalem in the Netherlands.

The main headings include—introduction; technique of smear-taking; technique of staining; examination by microscopy. Five thousand copies have been printed in English and arrangements are being made for its translation and printing in French, Spanish and Portuguese.

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Letters to the Editor

THE MONTHLY SUPERVISION OF ANTI-LEPROSY DRUGS IN THE AMAZONAS STATE OF BRAZIL

Sir,

I have recently returned to this country after working for 2 years in the Southern part of the State of Amazonas in Brazil. The State covers an area of 1,558,987 km^2 and the population at the end of 1981 was 1·5 million, of whom 15,767 were registered as having leprosy. The part in which I worked covered about 204,800 km^2 with a population of 140,000, amongst whom were 1487 cases of leprosy at the end of 1981—a figure which is acknowledged by local experts to fall far short of the real extent of the problem. Although we had some problems with accurate classification, my understanding is that approximately 38% of all new cases registered in 1981 were lepromatous or 'dimorphous' and under the latter term, many cases would conform to the B (borderline) classification of the Madrid system.

In view of the recommendations which have recently been made by the World Health Organization for multiple drug therapy in all forms of leprosy, including the supervision of monthly rifampicin in both pauci- and multi-bacillary patients, and clofazimine in multi-bacillary cases, I thought it might be worth recording that in the above area (and almost certainly in many parts of Brazil, and South America generally), we were extremely lucky to see our patients twice a year, despite constant travelling. In fact, under such conditions, and with various other problems concerning transport and the availability and distribution of drugs, we did not consider it wise even to attempt multiple drug therapy in the area described. One wonders how 'monthly supervision' is to be accomplished in other parts of the world, where distances are enormous and health services poorly developed? In the area where I was working it will be a long time before anything like monthly supervision becomes practicable. The development of health care structures and practical advice to resolve this sort of problem would be at least as valuable as some of the scientific and research projects which currently attract so much attention and money.

M N POWELL

25 Keswick House
Crawford Road, London SE5 9NL

IA-LIKE ANTIGENS IN LEPROSY

Sir,

Indira Nath in her review of the Immunology of Leprosy[1] states that 'Ridley, using formalin fixed tissues which may destroy [Ia-like] antigens, reported contradictory results showing a selective absence of [these] antigens on macrophages of lepromatous lesions.' We would point out that we did not use formalin fixative, but a fixative which is optimal for the preservation of antigens.[2] We did not suggest that the dendritic-type cells we observed were macrophages, nor did we claim to present the whole population of cells which can be shown to be Ia positive using cryostat material. Thus our results are complementary, not contradictory, to those of other workers. But we think the selectivity of our system has produced a significant result which has been missed by less selective systems.

MARIAN J RIDLEY and D S RIDLEY

Hospital for Tropical Diseases
4 St Pancras Way, London NW1 0PE

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Nath I. Immunology of human leprosy current status. *Lepr Rev* 1983; (special issue) 31S–45S.
Ridley MJ, Ridley DS. Unique expression of HLA-DR (Ia-like) antigen in the lesions of polar tuberculoid leprosy. *Lepr Rev* 1982; **53:** 249–52.

'SAVE OUR SOLES', J W BRANDSMA AND J G ANDERSEN

Sir,

In this most instructive article published in *Leprosy Review* (1983; **54,** 248–52) the authors, among others, advised that fingers can also be used for scraping the soles of the feet. This advice could be dangerous, especially when the hands are anaesthetic—and this is likely in patients with anaesthetic feet. The thickened sole can be very rough, cracked and sometimes have projecting scales that cut like a sharp blade. I once saw a surgeon cut his hand sufficiently to cause it to bleed on such a thickened sole.

I would, therefore, discourage the use of the bare fingers for scraping thickened soles. It is possible to save your soles and lose your fingers!

J K NSIBAMBI

ALERT
PO Box 165
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Errata in *Leprosy Review* Volume 55, Number 1, March 1984
We apologise for the following errors in this issue:

1 In '*The occurrence of leprosy in an eight-member family—a case report*' by Sarojini Peringali Aredath, the opening paragraphs (p. 47) should read as follows:

Case reports

1 *AL card no. SJO 7346, a female child born in 1974*, hailing from Ambo, Shoa Region, was brought to ALERT hospital on 21 August 1981 with multiple shiny nodules all over the face, ears, limbs and buttocks of 2 years' duration. The lesions increased 4 months before admission. The trunk was free of lesions.

There was no thickening of nerves or anaesthesia. A diagnosis of lepromatous leprosy of the histoid type was made. Skin smears revealed a Bacteriological Index (BI) of 4·8 and a Morphological Index (MI) of 7·8. The child was admitted and a course of rifampicin 300 mg daily for 3 weeks and dapsone (DDS) 100 mg daily was given.

2 *The father accompanying the child* was examined and found to have no clinical evidence of leprosy. He was unaware of any other cases of leprosy in the family or in his neighbourhood. His present wife, who is the mother of all the children in this report, was his third. He had had children by his first and second marriages, but no medical details were available. The child described above was the third child of the third wife and he was asked to bring the others for examination, together with the wife. On a subsequent attendance, he himself was re-examined and again found to be free of clinical signs, but to have a positive smear for acid-fast bacilli (AFB) at one site, namely the forehead, where the BI was 2+; it was negative at other routine sites. This positive finding was checked carefully and confirmed, and in view of the circumstances, he was treated with DDS 100 mg daily.

2 '*"Neural histoid." Histoid leproma in peripheral nerve: a case report*', by K. Ramanujam, S. Arunthathi, C J G Chacko & Mary Jacob, pages 63–68, Figure 1 should have carried the symbols M = months and W = weeks, and the original magnification for Figures 4 and 5 should have been recorded as × 400.

Editor

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Book Reviews

Properties of Monoclonal Antibodies Produced by Hybridoma Technology and their Application to the Study of Diseases, Proceedings of a Symposium held at the National University of Singapore, October 1981.

This is a paperback of 199 pages, produced by UNDP/World Bank/WHO for the Special Programme for Research and Training in Tropical Diseases, Geneva, and published in 1982. The six parts include: Preparation, properties and uses of monoclonal antibodies (MCA); MCA to protozoa; MCA to metazoa; MCA to bacteria and viruses; MCA to cell surfaces and immunoglobulins and finally, 'future outlook'. Part IV on MCA to bacteria and viruses includes papers on the production and characterization on MCA to *Mycobacterium leprae* (Gillis and Buchanan); MCA to *M. leprae* (Humber) and antigenic analysis of mycobacteria using MCAs (Pinto). [It is our understanding that *bona fide* applicants with a research or scientific interest in this subject may apply to the TDR programme in Geneva for a copy of this book. *Editor.*]

Monoclonal Antibodies in Clinical Medicine, by Andrew J McMichael and John W Fabre, Academic Press, London, 1983, 678 pages, price £34.00.

The publishers description reads as follows:

'This book, with an introductory chapter by Dr Cesar Milstein on the historical aspects and general principles of monoclonal antibodies, brings together comprehensive reviews on all the major areas of current clinical applications and speculates on future clinical uses. Arranged into 24 chapters, the book covers all the main areas of clinical research including oncology, haematology, microbiology, genetics, pharmacology, drug targeting, neurobiology and obstetrics. The use of monoclonal antibodies for diagnosis, prognosis, therapy and understanding of pathophysiology is discussed in each section. Of immediate importance is not only their use in the treatment of leukemias, graft rejection and drug overdose, but also the potential of monoclonal antibody/toxin conjugates as 'magic bullets'. In addition, the identification of possible tumour-specific antigens and microbial antigens which might form the basis of vaccines for some of the virally induced neoplasmas and infectious diseases provides an area of great potential clinical benefit.

In November 1982, Dr McMichael, who is University Lecturer in Medicine at the Nuffield Department of Clinical Medicine, University of Oxford, was awarded a Medical Research Council Clinical Research Professorship as MRC Clinical Research Professor of Immunology.'

Traditional Medicine and Health Care Coverage; a reader for health administrators and practitioners, edited by Robert Bannerman, John Burton and Ch'en Wen-chieh, WHO, 1983, 432 pp + index. Swiss fr. 35.

The purpose of this book is to provide a better understanding of traditional, indigenous and unorthodox systems. This should help to foster team work among all categories of health workers. The book therefore examines the most common patterns of these systems and some of their local or regional variations, and suggests how health practitioners and administrators might best apply this information as they endeavour to improve health care coverage, particularly in the developing countries. This book is illustrated and is available from Distribution and Sales, WHO, 1211 Geneva 27, Switzerland.

A Manual of Leprosy, edited by R H Thangaraj, The Leprosy Mission (India), 1983, 402 pp, hardback.

Edited by the Director of the Leprosy Mission for Southern Asia and published in New Delhi, the first edition (1976) was entitled 'Textbook of leprosy for students and para-medical workers'; the second (1980) under the present title. Twenty-two eminent contributors have cooperated to cover virtually the whole subject of leprosy from history (Chapter 1) to the National Leprosy Control Programme (Chapter 29). The book is profusely illustrated with black and white and also colour plates, together with diagrams and tables. Enquiries in India to Dr R H Thangaraj, The Leprosy Mission, 4th Floor, Sheetla House, 73–74 Nehru Place, New Delhi 110019; in the UK to Leprosy Mission (International) London.

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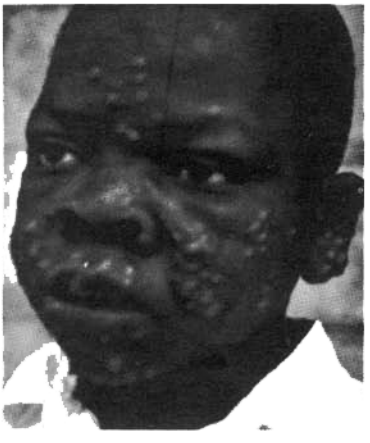

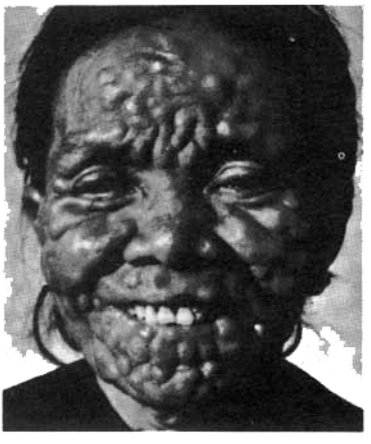

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1. Chemotherapy of leprosy for control programmes, Report of a WHO Study Group, WHO Technical Report Series 675, WHO, Geneva 1982.
2. S. J. Yawalkar, J. Languillon, S. K. Hajra, A. C. McDougall, S. Gosh, D. V. A. Opromolla, C. J. S. Tonello. Once-monthly Rifampicin plus daily Dapsone in initial treatment of lepromatous leprosy. Lancet 1199, 29 May 1982.

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