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Editorial

THE MOLECULAR BIOLOGY OF *MYCOBACTERIUM LEPRAE*

Introduction

Although *Mycobacterium leprae* was one of the first organisms to be established as causing human disease,[1] less is known about it than virtually any other bacterium of medical importance. This is because it has not yet been grown in bacteriological culture, and hence many of the approaches adopted for studying the basic biology and biochemistry of other bacteria are not applicable. To some extent this has been circumvented by the use of experimental animals. However, the limitations of working *in vivo*, and, in particular, the uniquely long generation time of *M. leprae*, even when growing optimally in the tissues of experimental animals, have proved major obstacles.

The recent application of a molecular biological approach to studying *M. leprae* is proving crucial in our basic understanding of the biology of the organism. Studies on its relationship to other bacteria, including mycobacteria, are now possible, while it is also possible to obtain large amounts of compounds, such as protein antigens or enzymes, which can be used to study interactions with the host at the molecular level. It should prove possible in the near future to use the basic information on the biology of *M. leprae* to devise strategies for circumventing the need to culture *M. leprae* for many clinical microbiological applications, such as drug-sensitivity testing, while further advances in understanding the basic biology of the organism should lead to a greater understanding of the host–pathogen relationship.

The molecular biological approach to studying *M. leprae*

The availability of large numbers of *M. leprae* from infected armadillo tissue provides access to *M. leprae* nucleic acids. The preparation of libraries of *M. leprae* DNA fragments in cosmid or bacteriophage *E. coli* vectors can serve as an inexhaustible source of *M. leprae* DNA.[2,3] By studying the nucleic acids and using molecular biological techniques much is being learned about the basic biology of *M. leprae*. In future, it seems likely that this knowledge will be put to practical use, and techniques which circumvent the need to culture the organism will be devised.

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THE TAXONOMIC POSITION OF *M. LEPRAE* BASED ON NUCLEIC ACID ANALYSIS

Mycobacteria belong to the high guanosine plus cytosine (G+C) subdivision of Gram positive bacteria, which also includes such actinomycetes as *Streptomyces*. Most mycobacteria have between 60 and 67 mole per cent G+C in their DNA, compared to 72% for streptomycetes, and 49% for *E. coli*. Interestingly *M. leprae* has a lower G+C% than other mycobacteria (56%; Clark-Curtiss *et al.*,[2] Imaeda *et al.*[3]), raising questions about its taxonomic position.

Using a molecular biology approach it is possible to address this. The 16S ribosomal RNA (rRNA) gene is convenient for studying phylogenetic relationships. rRNA is present in all free-living organisms; some stretches of the nucleotide sequence are variable, whereas others are conserved by functional constraints. The conserved sequences permit the alignment of the variable sequences; the degree of variation within these sequences is a reflection of the taxonomic and phylogenetic relationship between organisms. When applied to the mycobacteria, it is clear that the division between slow-growing and fast-growing species is a true phylogenetic division, that is, the division is clearly recognizable in terms of the 16S rRNA sequence,[4] and that *M. leprae* is correctly positioned in the slow-growing mycobacteria group.[5–7]

CLONING AND EXPRESSION OF *M. LEPRAE* GENES

One of the major areas of impact of molecular biology on the study of *M. leprae* is cloning of genes and high level expression to provide large amounts of protein. This has been widely applied for the production of recombinant proteins of immunological interest. This is a particularly attractive approach to use with an organism that cannot be grown in culture.

The approach was initiated by Young *et al.*,[8] who cloned small fragments of randomly sheared *M. leprae* DNA into the *E. coli* phage λgt11. In this vector, the cloned DNA is inserted into the coding region of the *E. coli* β-galactosidase gene, and the resulting protein is a hybrid of β-galactosidase and the *M. leprae* DNA-encoded protein. Expression of the *M. leprae* protein can then be detected immunologically, for example using monoclonal antibodies which have been raised against *M. leprae*. Once the *M. leprae* fragment has been identified, it is then possible to obtain the entire gene, to sequence it, and to manipulate high-level expression of the protein so that it can be purified and used for immunological studies.

This has now been carried out for a number of *M. leprae* proteins by a variety of different groups. In the initial experiments, mouse monoclonal antibodies were used to screen for expression of *M. leprae* antigens.[8] Characterization of the proteins recognized by the monoclonal antibodies revealed that many of them belonged to a well-characterized group of highly conserved proteins—heat shock proteins.[9,10] Several of these have now been expressed at high level and are being used to investigate their interaction with the immune response.

Alternative approaches for screening *M. leprae* expression libraries for immunologically important proteins have involved using sera from patients[11–13] or T cells.[14] These studies have identified proteins other than those selected by monoclonal antibodies.

Although the studies outlined above have demonstrated that *E. coli* is an excellent host for cloning and expression of mycobacterial proteins, for many applications using

mycobacteria themselves as cloning hosts is necessary. Many studies are now in progress to develop mycobacterial vectors so that experiments involving gene transfer between mycobacteria can be carried out.[15–20]

MOLECULAR METHODS FOR RAPID DETECTION AND IDENTIFICATION OF *M. LEPRAE*

There has been a great deal of interest in recent years in using molecular techniques to detect and identify microorganisms. Such techniques would be particularly attractive where the microbe in question could not be cultured, as with *M. leprae*. The polymerase chain reaction (PCR) is a technique used for amplifying small quantities of DNA (or RNA) to the point where they are readily visualized. The technique exploits two basic principles of biology; first that the affinity of single-stranded DNA for its complementary sequence is strong and specific, and secondly that it is possible to use one strand of DNA as a template to synthesize its complementary strand. In essence, the PCR involves the cyclical synthesis of DNA copies from a single template strand; the resulting increase in the number of copies of the template DNA is exponential, allowing, in principle, a single copy to become amplified so that it is readily detectable on a gel.

The reaction involves synthesizing copies of a region of DNA ('the target sequence') from oligonucleotide primers which bind to opposite strands of DNA and flank the target sequence. Each cycle of the PCR involves separating the strands of target DNA by heat denaturation, binding of the oligonucleotide primers to their complementary sequences within the target DNA ('primer annealing') and synthesis of new DNA between the primer sequences ('primer extension'). Each newly-synthesized DNA strand then becomes a template for the next cycle, so that after 20 or 30 cycles the original sequence has been amplified by approximately 10-million fold. The various stages of each cycle (denaturation, annealing and primer extension) occur optimally at different temperatures; by using a DNA polymerase which is not destroyed at high temperatures, the entire reaction can be performed in a single tube by simply changing the temperature on a cyclical basis, a process which can be achieved automatically using a microprocessor-controlled thermocycler.

PCR has been used to study DNA from fossils, to study the relatedness of different species of plants, animals and bacteria and in the forensic laboratory to identify criminals. The most obvious use in the clinical microbiology laboratory is in diagnosis and species identification, where its combination of exquisite sensitivity (minute quantities of DNA can be amplified to detectable levels) and specificity (the degree of specificity depends on the selection of primers; however, the starting material can be a complex mixture of host tissue and contaminating organisms) make the technique potentially extremely powerful.

A number of approaches have been described in which PCR has been used to detect and identify *M. leprae* in infected tissue. The main differences are at the point of DNA extraction and in the choice of target sequences. The latter consideration embodies the specificity of the technique—a target sequence with species-specific regions on which the oligonucleotide probes are based will provide a species-specific detection system. Some of the target sequences which have been used for the detection of *M. leprae* are shown in Table 1. PCR represents a powerful, sensitive and specific procedure which has been shown to work in experimental systems for detecting *M. leprae*. However, its role in the clinical diagnostic or clinical research laboratory is unclear. The current keen interest in the application of PCR technology for the detection of *M. leprae* should be pursued with critical objectivity.

Table 1. PCR *M. leprae* target sequences

Target sequence	Reference
Antigen encoding sequences	
18 kD	Williams *et al.*[21]
36 kD	Hartskeerl *et al.*[22]
65 kD	Woods & Cole[23]
Non-antigen encoding sequences	
Repetitive sequence	Woods & Cole[23]
Ribosomal RNA sequences	Cox *et al.*[6]

THE *M. LEPRAE* GENOME MAP

As discussed above, libraries of *M. leprae* DNA can serve as an inexhaustible source of material. However, a great deal of work is still required to obtain the piece of DNA of interest. The establishment of an 'ordered' library and a genetic map of the *M. leprae* genome[24] provides an important resource which should prove to be of great benefit to scientists working on the molecular biology and genetics of *M. leprae*.

Essentially, an ordered library involves 'fingerprinting' the cloned DNA fragments such that their relative position on the *M. leprae* chromosome can be determined. The position of known genes can then be located by hybridization with previously identified probes, and a comprehensive picture of the organization of the genome obtained. Currently an ordered library and map has been constructed, such that virtually complete coverage of the chromosome has been obtained and the position of 72 genetic loci determined.[24]

The value of such a library and map is that it provides an important resource for scientists interested in the molecular biology of *M. leprae*. For example, it has been possible to identify and study the gene encoding the target enzyme of rifampicin, the β subunit of RNA polymerase, making it possible to devise methods to rapidly diagnose rifampicin resistance (see below). This could be achieved with the minimum of effort because the locus had been identified using probes from *E. coli*.

THE MOLECULAR BASIS OF RIFAMPICIN RESISTANCE, AND RAPID DETECTION OF RESISTANT ISOLATES

Detection of rifampicin resistance currently takes between 3 weeks and 2 months for *M. tuberculosis* and between 6 and 12 months for *M. leprae*. Thus treatment is given empirically, without prior knowledge of the sensitivity status. Recently the molecular basis of rifampicin resistance of *M. leprae*[25] and *M. tuberculosis*[26] has been determined, and techniques for diagnosing resistance within hours, rather than weeks or months, are being developed.[26]

Resistance to rifampicin involves alteration of the RNA polymerase; because most *E. coli* rifampicin resistance mutations occur within a short stretch of DNA in the gene that encodes the RNA polymerase subunit β (*rpoB*), this part of the gene was concentrated on to define mutations in *M. tuberculosis* and *M. leprae*. For *M. tuberculosis* 98% of resistance mutations could be identified within a region of 23 amino acids,[26] while

all 9 isolates of rifampicin resistant *M. leprae* had mutations in the same site.[25] Telenti *et al.*[26] have used this information to devise a rapid test for rifampicin-resistant *M. tuberculosis*. Using a technique known as PCR–SSCP (Polymerase Chain Reaction–Single strand conformation polymorphism), they are able to distinguish rifampicin resistant isolates of *M. tuberculosis* within hours, rather than weeks. A similar approach could be used for the rapid detection of rifampicin resistant *M. leprae*.

Conclusions

Although the underlying problems of studying *M. leprae* in the laboratory remain, i.e. it still cannot be cultured *in vitro*, and takes many months to grow in laboratory animals, the availability of large amounts of organisms from the armadillo have enabled studies on the biochemistry, immunochemistry and molecular biology of *M. leprae* to move forward. I have not attempted, in this brief review, to document all the facts known about the organism but have tried to emphasize the sort of approaches which are now available for studying the basic biology of the organism. By using the examples of PCR and rifampicin resistance, I have tried to illustrate how these basic studies might provide the basis for developing techniques which are useful in the clinical laboratory and circumvent the need to culture the organism.

There is a great deal of (justified) optimism about the success of MDT and the potential for control and elimination of leprosy. However, our experience with tuberculosis emphasizes that this optimism should be tempered with caution; in spite of the fact that effective chemotherapy against *M. tuberculosis* has been available for several decades, TB is still a major public health problem in developing countries, and the spectre of increased incidence and multiple drug resistance in industrially developed countries is now emerging.[27] Although prospects for eradicating leprosy are now brighter than at any other time, *M. leprae*, like *M. tuberculosis*, may yet have some surprises in store.

Acknowledgment

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Serum lactoferrin in lepromatous leprosy patients

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Summary The serum concentrations of lactoferrin were determined by competitive enzyme immunoassay in the sera of 38 lepromatous leprosy patients and 16 healthy volunteers. Of the 38 lepromatous patients, 25 were without any sign of reactions while 13 were suffering from ENL type of reactions. The lactoferrin levels, in both types of patients, were observed to be significantly higher ($P < 0{\cdot}01$ and $< 0{\cdot}001$, respectively) than in that of healthy volunteers. The rise in lactoferrin level in reactive patients was also higher ($P < 0{\cdot}05$) when compared to those without reactions. The serum lactoferrin levels were also found to be associated with bacterial load ($r = 0{\cdot}414$; $P < 0{\cdot}01$) indicating that in lepromatous leprosy patients, lactoferrin may not be very effective in preventing the growth of *Mycobacterium leprae*. Further studies to improve the understanding of the role of elevated levels of lactoferrin in pathogenesis of lepromatous leprosy patients and in establishing its possible use in predicting the occurrence of ENL type of reactions would be worthwhile pursuing.

Introduction

Leprosy, a chronic disease caused by *M. leprae*, is a spectral disease that ranges from tuberculoid leprosy on one hand to lepromatous leprosy on the other. Patients suffering from the lepromatous type of leprosy lack any immunity against *M. leprae* which, therefore, can unrestrictedly multiply inside the body. Due to the dissemination of *M. leprae* into various parts of the body, a host of components of the immune system are influenced, giving rise to a variety of immuno-biochemical changes, including a rise in the immune complex level.[1–6] However, there has never been a report on the status of lactoferrin, an iron binding protein,[7,8] in leprosy patients. Since *M. leprae* and immune complexes are known to interact with polymorphonuclear leucocytes[9–11] (the main lactoferrin producing cells),[12,13] and accumulation of polymorphonuclear leucocytes is a salient feature of erythema nodosum leprosum (ENL) lesions,[14] we felt it important to determine the levels of lactoferrin in sera from lepromatous leprosy patients with and without ENL. As far as we know, this is the first study on this subject.

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Materials and methods

SERA SAMPLES

Sera samples were collected from lepromatous leprosy (LL) patients who attended the out-patient department of the Central JALMA Institute for Leprosy, Agra, India. Although most of the patients were under treatment they all showed an active form of the disease; 25 patients were without any sign of lepra reaction while 13 patients were having ENL type of reactions. The sera collected from 16 normal healthy individuals formed controls for the study. The 3 groups were matched for age and sex. All the sera collected was stored at $-70°C$ until used.

REAGENTS

Bovine-serum albumin, lactoferrin and tween-20 were purchased from the Sigma Chemical Company, St. Louis, U.S.A. Sheep anti-human lactoferrin antibody (Ig fraction) was purchased from Serotec, Oxford, U.K. and peroxidase conjugated rabbit anti-sheep antibody (affinity pure IgG) was collected from Jackson Immuno-research Lab, Inc., Baltimore Pike, West Grove, PA19390, U.S.A. All the other chemicals used for making the buffers were of reagent grade.

BACTERIOLOGICAL INDEX

The bacteriological status of leprosy patients was studied by determining the bacterial index (BI). Slit-skin smears were taken from 4 sites, the left and right lobes of both the ears, and 2 representative skin lesions. The smears were stained according to Ziehl–Neilson's method. The average bacterial density was calculated following the Ridley's logarithmic scale.[15]

MEASUREMENT OF SERUM LACTOFERRIN

The lactoferrin levels in the sera samples were measured by competitive enzyme linked immunosorbent assay (ELISA). For this assay lactoferrin was diluted to 2·0 μg/ml in carbonate buffer (pH 9·6) and 100 μl of this suspension was added to each well. The plates (Nunc, Denmark) were kept at 4°C overnight for coating. After this, the wells were washed 3 times with phosphate-buffered saline supplemented with 0·1% tween-20 (PBST). Excess binding sites, for non-specific binding of proteins, were blocked at 37°C for 2 hr, with 1% BSA (200 μl/well) prepared in PBST. Next, 50 μl of the undiluted serum sample was added to each well and a
lactoferrin antibodies, diluted (1:5000) in PBST. For each test at least 3 wells were used. The plates were then kept at 37°C for 2 hr, washed, dried and further incubated for 45 minutes with peroxidase labelled anti-sheep antibodies diluted (1:5000) in PBST. After washing the plates, the colour in the wells was developed by adding 100 μl/well of the substrate (1,2-orthophenylenediamine dihydrochloride) for 20 minutes. Finally, the enzymatic reaction was stopped by 50 μl/well of 7% H_2SO_4. The optical density values were read by using an ELISA reader (Titertek, Multiscan, Flow Laboratories, U.K.). The values of serum lactoferrin were calculated using a standard curve obtained with purified lactoferrin.

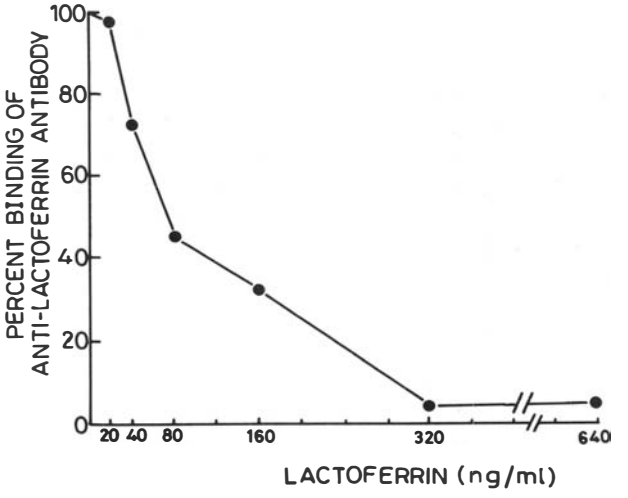

Figure 1. Standard curve for lactoferrin.

Results

A standard curve was obtained on graph paper by measuring the percent binding (in the presence of several concentrations of the purified lactoferrin) of anti-lactoferrin antibodies to the lactoferrin coated wells. The binding of anti-lactoferrin antibody, in the absence of purified lactoferrin, was considered as 100%. A dose-dependent, inverse relationship between the lactoferrin level (up to 320 ng/ml) and the binding of anti-lactoferrin antibody was obtained (Figure 1). Thus less binding of anti-lactoferrin antibody indicated higher concentration of lactoferrin, and vice versa.

Lactoferrin levels in the sera of the controls and the lepromatous leprosy patients were measured using competitive ELISA. Figure 2 depicts the lactoferrin levels in sera from each subject. The range of lactoferrin levels in the sera from the controls was 0·32–1·58 μg/ml, in lepromatous leprosy patients without reactions it was 0·44–5·58 μg/ml, while in those having ENL type of reactions it varied from 1·08 to 6·24 μg/ml. The mean ($\pm$S.D.) lactoferrin level ($3{\cdot}25 \pm 2{\cdot}01$ μg/ml and $1{\cdot}95 \pm 1{\cdot}47$ μg/ml) in the sera of both types (reactive and non-reactive, respectively) of lepromatous leprosy patients were significantly higher ($P < 0{\cdot}001$ and $P < 0{\cdot}01$) than that ($0{\cdot}99 \pm 0{\cdot}46$ μg/ml) of the controls. We also found that lactoferrin in reactive lepromatous patients was higher ($P < 0{\cdot}05$) compared to non-reactive leprosy patients. Further analysis showed that lactoferrin concentration in 28% (7 out of 25) LL patients were below the mean level of the controls, whereas all ENL patients showed above the controls' mean value.

In order to better understand the anti-*M. leprae* activity of lactoferrin in lepromatous leprosy patients, the correlation between the lactoferrin level and the bacterial index was also investigated. A positive correlation ($r = 0{\cdot}414$; $P < 0{\cdot}01$), though weak, was noted between the lactoferrin level and bacterial load (Figure 3).

Discussion

The present study demonstrates, for the first time, elevated concentrations of lactoferrin

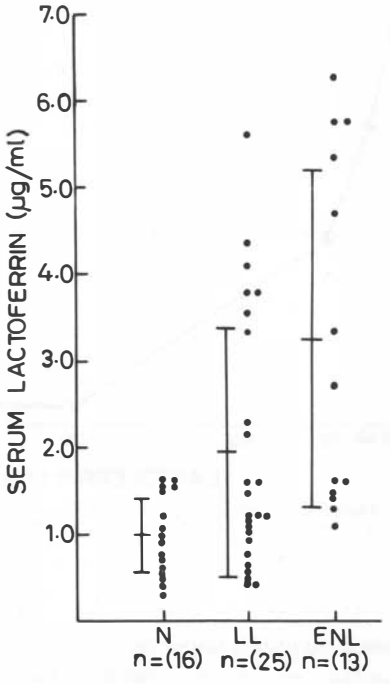

Figure 2. Lactoferrin levels in sera from lepromatous leprosy patients without reactions (LL), from LL patients suffering from erythema nodosum leprosum (ENL) type of reaction and from healthy controls (N). Vertical bar indicates mean ± S.D. for each group.

in lepromatous leprosy patients as a group. However, there were patients whose lactoferrin was below the mean value of the controls. The individual response to chemotherapy and the genetic make up of these patients might cause this. It is noteworthy that the lactoferrin level was found to be still higher in ENL cases than those without ENL. From these findings it appears that a continuous rise of lactoferrin level in lepromatous patients might prove to be helpful in predicting the occurrence of ENL type of reactions. To confirm this theory, a follow-up study of lepromatous patients is required. It would also be interesting to know whether lactoferrin has any role in the pathogenesis of lepromatous leprosy, and even more interesting if it affected ENL patients.

There are some possible mechanisms to explain the increased lactoferrin production in lepromatous leprosy patients. Polymorphonuclear leucocytes (PMNs) are an important component of the immune system and contain lactoferrin in their specific storage granules.[12,13] On their activation by a variety of stimuli, for instance the phagocytosis of microbes[16–18] or interaction with immune complexes,[10,11] the lactoferrin from these granules is discharged both in phagosomes and to the external environment. Phagocytosis of *M. leprae* by PMNs has been described in lepromatous leprosy patients[9] and it has also been demonstrated that the immune complex level is elevated.[1,4] It is possible that elevation in the levels of serum lactoferrin in lepromatous leprosy patients may be caused by the stimulation of PMNs by phagocytosed *M. leprae* and immune complexes. The higher level of lactoferrin in reactional patients could be attributed to their higher load of

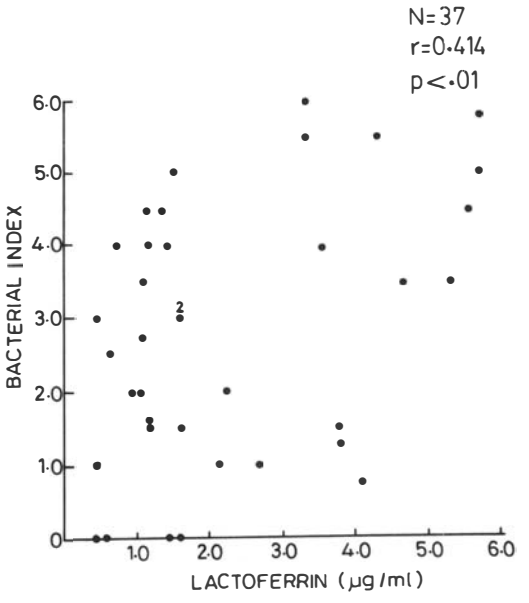

Figure 3. Correlation of lactoferrin levels with bacterial index in lepromatous leprosy patients.

immune complexes present, compared to patients not suffering from reactions.[4] Further, the spillover during tissue necrosis due to lysis of PMNs accumulated at the site of the reactive lesions[14] might also add to the lactoferrin level in ENL patients.

Evidence shows that lactoferrin mediates the antibacterial activity against a wide range of micro-organisms, including Gram negative and Gram positive bacteria.[7,8,19,20] Some of the mechanisms involved in antibacterial action of lactoferrin include the impedency of iron utilization by bacteria, interfering with the metabolism of microbial cells, destabilization of the microbial cell wall, and stimulation of intracellular killing of micro-organisms by phagocytes, etc.[7,8] In the present study a positive correlation was observed between *M. leprae* load and lactoferrin levels which appears to suggest that lactoferrin is unable to prevent bacterial multiplication inside the host.

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Lactoferrine sérique chez les sujets lépreux lépromateux

O. Parkash, B. K. Girdhar et U. Sengupta

Résumé Nous avons déterminé les concentrations sériques de lactoferrine par test immunoenzymatique par compétition, dans le sérum de 38 sujets lépreux lépromateux et de 16 volontaires en bonne santé. 25 des 38 sujets lépromateux n'ont présentés aucun signe de réaction tandis que 13 ont souffert de réactions du type ENL. Chez ces deux types de sujets nous avons observé que les concentrations de lactoferrine étaients significativement plus élevées ($P < 0{,}01$ et $< 0{,}01$ respectivement) que chez les volontaires en bonne santé. La hausse du taux de lactoferrine était également plus élevée ($P < 0{,}05$) chez les sujets qui ont présenté des réactions que chez ceux qui sont restés sans réaction. Nous avons également observé que les concentrations sériques de lactoferrine étaient associées à la charge bactérienne ($R = 0{,}414$; $P < 0{,}01$) ce qui indique que chez les sujets atteints de lèpre lépromateuse, la lactoferrine n'est peut-être pas très efficace pour empêcher la croissance du *Mycobacterium leprae*. Il y aurait lieu de faire d'autres études pour comprendre le rôle des concentrations élevées de lactoferrine dans la pathogénie de la lèpre lépromateuse et pour déterminer son utilisation possible dans la prévision de l'occurence des réactions de type ENL.

Lactoferrina del suero en pacientes de lepra lepromatosa

O. Parkash, B. K. Girdhar y U. Sengupta

Resumen Mediante el inmunoensayo de enzimas competitivas se determinaron las concentraciones de lactoferrina en el suero de 38 pacientes de lepra lepromatosa y 16 voluntarios sanos. De los 38 pacientes lepromatosos, 25 no presentaron signos de reacciones, mientras que 13 pacientes sufrieron reacciones de tipo ENL. Los niveles de lactoferrina observados en ambos tipos de pacientes fue significativamente superior ($P < 0{,}01$ y $< 0{,}01$, respectivamente) que el de los voluntarios sanos. El aumento del nivel de lactoferrina también fue superior en los pacientes reactivos ($P < 0{,}05$) comparado con el de los pacientes no reactivos. También se observó que los niveles de lactoferrina en el suero están relacionados con la carga bacteriana ($r = 0{,}414$; $P < 0{,}06$), lo cual señala que en los pacientes de lepra lepromatosa la lactoferrina tal vez no sea un medio muy eficaz para prevenir el aumento de *Mycobacterium leprae*. Bien valdría la pena efectuar mayores estudios para comprender el papel que desempeñan los niveles elevados de lactoferrina en la patogénesis de los pacientes de lepra lepromatosa, y para establecer su posible uso en el pronóstico de la incidencia de reacciones de tipo ENL.

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Field trials on the use of *Mycobacterium w* vaccine in conjunction with multidrug therapy in leprosy patients for immunotherapeutic and immunoprophylactic purposes

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Summary A double blind field trial was started with a candidate anti-leprosy vaccine, *Mycobacterium w* as an immunotherapeutic and immunoprophylactic agent against leprosy in a highly endemic region with a prevalence rate of over 18 per 1000 population. By 31 August 1992, 224 villages have been surveyed, covering a population of 307,981 (1981 census). A total of 979 MB patients and 2801 PB patients have been registered. A total of 19,453 household contacts of leprosy patients have been examined for clinical signs of disease, of which 16,519 have received the initial dose while 10,434 have also received the booster dose of vaccine/placebo. The aims and objectives, study design of the trial, present status as well as the socio-cultural aspect involved are highlighted in this paper.

Introduction

A potential leprosy vaccine, based on a cultivable, rapid growing, non-pathogenic bacillus, *Mycobacterium w* (*M.w*), was proposed by Talwar *et al.* in 1978.[1] This bacillus was identified by its ability to elicit cell-mediated immune reactions similar to those evoked by *M. leprae* with cells from tuberculoid leprosy patients.[2–5] It had, in addition,

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antigens that evoked *responses* from cells of lepromatous leprosy patients, which otherwise have poor blast transformation and cytokine production with *M. leprae*. The presence of B- and T-cell determinants on *M.w*, common to *M. leprae*, has recently been confirmed by other criteria.[6,7] *M.w* is the code word by which this bacillus was investigated experimentally. Though it has growth and metabolic characteristics similar to mycobacteria currently listed in Runyon Group IV, it differs from them, in its biochemical properties, in several respects—it can be distinguished from *M. phlei* and *M. vaccae* by being non-pigment producing, and *M.w* is urease negative, in contrast to *M. fortuitum*, *M. smegmatis* and *M. chilae* which are urease positive. Furthermore *M.w* is distinguishable from *M. smegmatis* by sugar fermentation and lack of utilization of acetamide as sole nitrogen source and arabinose and fructose as sole carbon source.[8,9] The proof that *M.w* is a unique strain was obtained by identification of a signature sequence in the highly conserved 5′ coding region of 65 kd antigen gene. *M.w* DNA was amplified using primers TB1-5′GAG ATC GAG CTG GAG GAT CC and TB2-5′AGC TGC AGC CCA AAG GTG TT, as previously described.[10] The amplified product DNA of 383 bp in size was cloned and sequenced. Comparison of the sequence with *M. bovis* BCG, *M. avium*, *M. paratuberculosis* and *M. fortuitum* which represent other groups revealed a specific signature sequence at bp position No. 121 (T instead of C) and at bp No. 130 (C instead of G), suggesting *M.w* as a unique strain (if not a unique species) of mycobacteria (Khandekar *et al.*, personal communication).

The vaccine consists of autoclaved suspensions of *M.w* in sterile saline. After due completion of toxicology studies, drug regulatory and ethical approvals, the vaccine has undergone Phase I and Phase II clinical trials. It is being assessed for comparative immunoprophylactic properties with the WHO sponsored vaccine, consisting of live B.C.G. and killed *M. leprae* in the Chengalpattu District of South India. In that trial *M.w* vaccine is being administered to about 35,000 members of the general population, in contrast to our trial where only high-risk household contacts of leprosy patients have received *M.w* vaccine. Thus the results of the trial at Chingalpattu will supplement our knowledge of the efficacy of *M.w* as an immunoprophylactic agent in the general population. The vaccine has also shown important immunotherapeutic effects in controlled trials conducted on active multibacillary (MB) leprosy patients where it was given as an adjunct to chemotherapy in the test group in 2 hospitals in New Delhi. Inclusion of the vaccine resulted in a faster bacterial clearance and the hastening of clinical recovery.[11]

In some patients the effect was dramatic—a lepromatous leprosy (LL) patient with a bacteriological index (BI) of 6+ showed bacteriological negativity and clinical inactivity after 15 months of chemo-immunotherapy.[12] Similar results have been obtained on a larger series of over 300 patients on whom the code has been recently opened. Another important histological action of the vaccine is the clearance of dermal granulomas. A statistically significant number of multibacillary (MB) patients (78·84%) given immunotherapy with *M.w* vaccine demonstrated either an upgrading or a clearance of granuloma from the lesions.[13] These studies underscore the important role that the vaccine can play in the treatment of leprosy. Another feature of vaccination with *M.w* was the conversion of about 80% of lepromin negative MB patients to lepromin positivity status.[14]

Immunotherapeutic trials with the vaccine gave highly satisfactory results, suggesting the wider use of the vaccine in leprosy control programmes. Shortening of the recovery period implies savings on the cost of drugs and medical care. A quicker fall of B.I. would

also lessen the infection load and be of benefit to the community. A major question arose as to whether inclusion of the vaccine in the regime approved by WHO for MDT, which is adopted in the National Leprosy Eradication Programme (NLEP), is feasible in the field. A trial for this purpose was approved by an expert group under the chairmanship of the Director of Indian Council for Medical Research (ICMR) and by the Drug Controller of India. This communication gives the protocol of this trial and also reports the progress achieved in its implementation over the last 2 years. We also discuss our strategies to attain a high compliance rate amongst vaccinees.

In trials combining immunotherapy with chemotherapy, another issue investigated is the immunoprophylactic benefit, if any, to household family members and contacts of leprosy patients.

Analysis of the results of vaccination in the present trial will be carried out at the end of 3 years from the start of the study, and again at the end of 6 and 9 years. Comparison between these 3 re-surveys will give a definite indication of the effect of vaccination on the incidence and prevalence rates of leprosy in the study area.

Aims and Objectives

The aims of the trials are to confirm:

1 the immunotherapeutic efficacy of the *M.w* vaccine under field conditions in MB leprosy patients when administered in conjunction with MDT, in terms of clinical improvement and bacterial clearance in comparison with MDT alone;
2 the incidence of reactions and their management in field conditions;
3 the immunoprophylactic effect of the vaccine in the contact population of both multi- and paucibacillary cases of leprosy in an endemic area;
4 the trend of leprosy in the study area under various modes of treatment with respect to incidence and prevalence rates; and
5 the benefits, if any, of including the vaccine with the present MDT for control of leprosy.

Study Area and Trial Size

The pre-MDT surveys conducted by the NLEP in 1988–89 recorded the prevalence rate of leprosy in the rural Leprosy Control Unit (LCU) of Ghatampur, within the district of Kanpur Dehat in the North Indian state of Uttar Pradesh, to be 18·19/1000 inhabitants. This region was selected to synchronize with the initiation of MDT in this LCU. In choosing a rural settlement, a consideration was that the population would not be migratory in large numbers over the observation years.

The trial size was calculated based on the following statistical presumptions:

(a) that the proportion of new cases (both MB and PB) in the contact population was 2·5 times higher than the proportion of new cases in the general population;
(b) that the initial incidence rate of leprosy in the general population was 10% of the prevalence rate; and
(c) that the percentage of dropouts during the follow-up period would be around 30%.

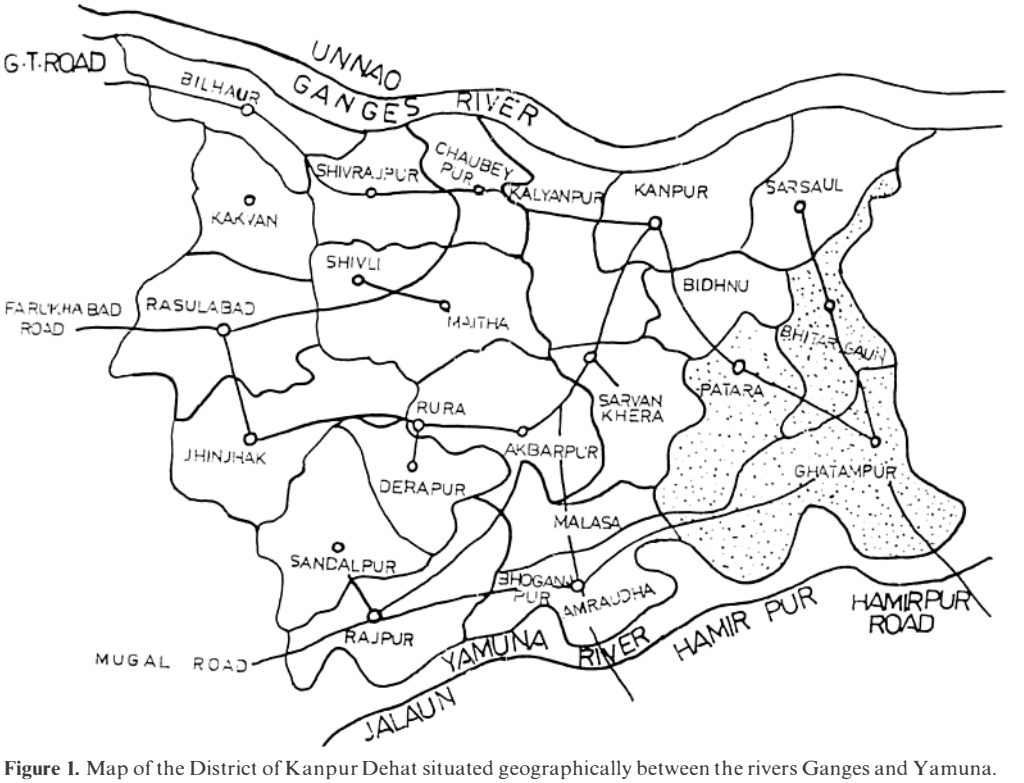

Figure 1. Map of the District of Kanpur Dehat situated geographically between the rivers Ganges and Yamuna. All the Community Blocks of the District are shown with the shaded portion depicting the 3 Community Blocks—Ghatampur, Patara and Bhitargaon—which form the study area for the present trial. These 3 Community Blocks have a population of 362,000 (1981 census) with a prevalence rate for leprosy of 18·19 per 1000 population.

A desired reduction in the proportion of new cases—i.e. a vaccine efficacy of 60% for a 10% level of significance (1-tailed test) and an 80% power of significance reckoned the trial size as 366,704 people.

The shaded area of Figure 1 shows the geographical location of the 3 community blocks within the LCU of Ghatampur with a total population of 362,000 (1981 census), which is almost the same as the calculated trial size for the study.

Study Group and Double Blind Coding

According to the trial protocol, the vaccine was to be administered to MB patients (LL, BL and BB types of leprosy) and to contacts of both PB and MB patients who are the high risk group for developing leprosy. Both MB and PB patients received MDT in accordance with the NLEP schedule.

Since the prevalence rate of leprosy in the community under study was high, each village in the block had an almost similar risk of leprosy occurrence. With this in view, the villages were divided on a purely random basis into 'experimental' and 'control' categories. Individuals in the experimental villages received *M.w* vaccine, while those in

the control villages received the placebo. For immunotherapy, the vaccine dose was 1×10^9 killed bacilli in 0·1 ml of saline. The vaccine was given intradermally in the left deltoid region. Subsequent injections contained 5×10^8 killed bacilli given at intervals of 3 months up to a total of 8 doses, alternating between the right and left deltoid areas. The placebo consisted of $\frac{1}{8}$ dose of the standard tetanus toxoid, dispensed in an equivalent volume (the idea was to provide a full dose of tetanus toxoid at the end of 8 injections). The initial doses of the vaccine/placebo were dispensed in clear vials, while the booster doses were dispensed in coloured vials. Each vial has a volume of 1·2 ml and provides up to 10 vaccine/placebo doses. The utilization efficiency ranged from 75% to 80%. For immunoprophylaxis, individuals in the experimental villages who were healthy family contacts of both MB and PB patients received *M.w* vaccine—2 doses of the vaccine were injected at 6-month intervals.

The target population was thus divided into 4 groups as follows:

Group	*Patients*	*Healthy contacts*
I	MDT + placebo	placebo
II	MDT + placebo	*M.w* vaccine
III	MDT + *M.w* vaccine	placebo
IV	MDT + *M.w* vaccine	*M.w* vaccine

Comparison between groups I and II will indicate the immunoprophylactic effect and that between groups I and III the immunotherapeutic effect, whereas the group I and group IV comparison will indicate the effect of combined immunoprophylactic and immunotherapeutic treatment.

Vials containing the vaccine and placebo were coded in a double-blind manner by the Institute for Research in Medical Statistics of the Indian Council of Medical Research (ICMR), New Delhi. The codes are kept with the ICMR. There are 8 types of vials, which can be distinguished from the first 2 characters of the code printed on the vials. Vials P1 to P4 are meant for immunotherapy while vials C1 to C4 are meant for immunoprophylaxis. The subsequent numbers after the first 2 characters denote the serial number of the vials within each type—e.g. Vial No. P1/117 denotes that the vial contains vaccine/placebo meant for immunotherapy to patients in the first group and is the 117th vial in this group.

All the villages in the trial area were stratified on the basis of prevalence rate of leprosy and the population of the villages to ensure comparable numbers of patients and contacts in all the 4 groups. Coding was done at the village level and since each village was to be allocated to 1 of the 4 groups, villages in each of the stratification categories were divided into clusters of 4 and then randomly allocated to 1 of the 4 groups with the help of a random number table.

Organizational and Operational Aspects

The trial is being jointly run by the NLEP Division of the Directorate General Health Services, Uttar Pradesh, and the field unit of the National Institute of Immunology, New Delhi. The organizational setup of the NLEP has been utilized for purposes of vaccination, and active support from the non-medical assistants (NMA) in the NLEP team helped the vaccination process. The baseline data on index cases of leprosy, and their addresses, were made available by the NLEP Unit, Uttar Pradesh, on the basis of the pre-

MDT survey carried out in 1988–89, and these data were used for the initial survey and vaccination. The villages are visited by the vaccinating team, along with the NMA catering to these particular villages, on days that do not clash with the primary duty of drug distribution. Before vaccinating anyone in a particular village, consent of the village headman, who is elected to represent the village, is obtained in a written format. The headman is made aware of the morbidity of the disease and of the potential benefits of the vaccine, and he is also told that the inhabitants might be receiving an injection which would be helpful against tetanus. A complete house-to-house survey is carried out as far as possible, and patients already registered with the NLEP are examined. The potential benefits of the vaccine are explained to these patients and their household contacts then they are all examined clinically, and only then is the vaccine administered.

Data Maintenance

Each patient (MB or PB) is registered in a separate proforma and given a number. There are separate series of numbers for MB and PB patients. There is provision in the proforma for recording the double-blind code group as well as the Circuit where the village the patient lives in falls As an example, an MB patient, Birender, from the village of Tikwapur of the coded group I belonging to Circuit C was registered as the 928th MB patient. His individual number is recorded as C928/I, the 1st letter indicating the circuit and the next 3 digits the MB patient number and the last Roman digit after the slash indicating the Group where that village falls.

The proforma also gives details of the eligible contacts of and their relationship to the index case. Most of the information generated in the field is recorded in the proforma. These proformas are then handed over to the statistics section of the field unit where data entry and verification is calculated using a Personal Computer based system. A detailed computer program package has been developed to facilitate analysis and consistency checking. This package includes programs that analyse changes in clinical status using Ramu's clinical scoring system,[15,16] as well as lepromin conversions, bacterial indices fall and histopathological trends, all of which are monitored at regular intervals in the MB patients.

Progress of the Trial

By 31 August 1992, 224 villages with a population of over 300,000 had been covered. A total of 3060 leprosy cases (979 MB and 2081 PB) were detected in this population. Table 1 gives the break-up of these cases into various groups and villages—24·7% were of the MB type. The prevalence rate was around 10/1000 population, which works out to be lower than the record of the initial survey carried out in this district before initiation of the MDT programme.

The exact number of migrating patients cannot be known exactly, as this information was not volunteered by the villagers. However, our survey demonstrated that a number of cases included in the pre-MDT survey list of NLEP were not true cases of leprosy. These cases were, for example, psoriasis, tinea, nevi and vitiligo. Furthermore, we detected 765 leprosy cases which were not originally counted in the NLEP survey. These could either be

Table 1. Trial coverage as on 31 August 1992

Group (code)	Number of villages	Population (1981)	Total cases seen		New cases seen*	
			MB	PB	MB	PB
P1, C1	54	77,819	237	789	27	177
P2, C2	58	73,024	251	678	37	141
P3, C3	65	87,901	242	665	26	124
P4, C4	47	69,237	249	669	49	184
Total	224	307,981	979	2801	139	626

* Leprosy cases not figuring in the pre-trial NLEP Survey.

cases which developed after the initial survey was conducted in 1988, or they were missed at the original survey, as this was conducted primarily by non-medical persons, even though trained in leprology. (Another reason could be that even after detection these cases had failed to turn up when called for registration for MDT.)

Vaccine or placebo was given to all active MB cases and will be repeated at 3-month intervals for 2 years. The 19,453 contacts of all leprosy cases, MB or PB, were all eligible to be given the vaccine/placebo for immunoprophylaxis. It was possible to immunize 16,519 (84·9%) contacts. The break-up as per coded groups is given in Table 2. The booster dose of immunoprophylaxis at 6 months was given to 10,434 contacts and it is expected that all contacts immunized initially will be given the booster dose. The new patients detected by us were registered by the NLEP team and MDT was given to them. The trial was therefore helping the NLEP to discover more cases and thus reduce the reservoir of infection. Similarly we also recorded the new cases detected by the NLEP team and vaccination was provided to them and their household contacts. The 2 teams were thus complementing each other for the ultimate benefit of the general population.

Reactions to vaccination

There was no clinically apparent systemic reaction to vaccination in either patients or their contacts. However, there was an instance of a hypersensitivity reaction with a generalized maculopapular, erythematous eruption. This case was treated with a short course of systemic steroids and antihistaminics which resulted in subsidence of the rash.

Table 2. Contacts of leprosy patients vaccinated

Group	Eligible contacts	First dose	Booster dose
C1	5212	4435	2520
C2	5075	4204	2859
C3	4422	3855	2756
C4	4744	4025	2229
Total	19,453	16,519	10,434

Table 3. Number of patients experiencing leprosy Types I and II reactions

	Total reaction cases*		Onset post-vaccination	
Group	Type I	Type II	Type I	Type II
P1	9	6	1	2
P2	8	3	4	1
P3	12	8	0	3
P4	13	8	6	1
Total	42	25	11	7

* Amongst all MB patients registered in the study.

At the site of the intradermal vaccination there developed in 1 week an erythematous papule or nodule, which healed in 3–4 weeks, giving rise to a healthy scar. Due to scratching of the site a secondary infection developed in about 3%. This was because of a lack of personal hygiene. A short course of local and/or systemic antibiotics was given in such cases.

Amongst the patients, there was a more or less similar incidence of Types I and II leprosy reactions in all 4 groups (Table 3) with 1–2 patients in each group developing neuritic reactions. These patients were detected early and given treatment with aspirin as well as other nonsteroidal anti-inflammatory (NSAID) drugs to prevent neurological deficits; 3 cases with non-healing plantar ulcers had to be admitted into the district hospital.

Comments

The progress of the trial so far supports combining a vaccine with standard MDT in the field. Logistically, the administration of the vaccine as immunotherapy once every 3 months to MB patients can be attained by co-ordination with the delivery of drugs by the staff of the NLEP unit. We have observed that the paramedical personnel were competent to deliver the vaccine correctly after a brief exposure and training.

The household contacts of the patients were well-motivated to accept the vaccine. Although the villagers, especially the young children, had a fear of injections, and some of the adults were apprehensive of the effect that vaccination would have on their daily work, this was overcome by informing them of the potential benefits and reassurance.

The non-medical assistants of the NLEP staff proved to be the main motivators for the vaccination, at least initially. They were the people who themselves reside in the villages and thus are trusted by the residents. Furthermore, most of the villages were deprived of the services of a doctor in the Primary Health Centre, thus the presence of doctors in our vaccinating team and the provision of free drugs for minor ailments and suitable advice for major ones went a long way to instil confidence in the villagers. This news spread by word of mouth to neighbouring villages so that when our teams visited them we were better received and the vaccination compliance increased appreciably as the trial progressed. Assistance was also extended by the village elder or the headman who also

convinced the villagers of the potential benefits of the vaccine. Gradually acquainting ourselves with the local dialect also helped.

We were able to immunize 80% of the eligible subjects in this category according to the protocol. The compliance rate would have been even higher, if the team had had the time to wait for the whole day, as some members of the family were unavailable as they had gone for work far away and others had often not returned from their fields before the team had to move on to the next village.

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Lepr Rev (1993) **64,** 302–311

Etudes sur le terrain sur l'emploi du vaccin *Mycobacterium w* en conjonction avec un traitement médicamenteux combiné chez les lépreux, à des fins immunothérapeutiques et immunoprophylactiques

R. Walia, K. G. Sarathchandra, R. M. Pandey, S. K. Parida, S. A. Zaheer, H. K. Kar, A. Mukherjee, R. Mukherjee et G. R. Salwar

Résumé Une étude à double aveugle sur le terrain a été entreprise avec le vaccin anti-lèpre à l'étude, *Mycobacterium w* comme agent immunothérapeutique et immunoprophylactique contre la lèpre dans une région fortement endémique dont le taux de prévalence est de 18 habitants sur 1000. Au 31 août 1992, 224 villages avaient été recensés et recouvraient une population de 307.981 habitants (recensement de 1981). Un total de 979 sujets MB et de 2801 sujets PB ont été inscrits. Un total de 19,453 personnes appartenant à l'entourage domestique des sujets lépreux, ont été examinées afin de détecter des signes cliniques de la maladie. La dose initiale a été administrée à 16.519 de ces personnes et la dose de rappel de vaccin/placebo à 10.434 d'entre elles. Dans ce rapport nous soulignons les buts et les objectifs, la conception de l'étude, la situation actuelle ainsi que les facteurs socio-culturels impliqués.

Estudios de campo en el uso de vacuna *Mycobacterium w* en forma conjunta con terapia de drogas múltiples en pacientes de lepra, para fines inmunoterapéuticos e inmunoprofilácticos

R. Walia, K. G. Sarathchandra, R. M. Pandey, S. K. Parida, S. A. Zaheer, H. K. Kar, A. Mukherjee, R. Mukherjee y G. R. Salwar

Resumen Se inició un estudio de campo doble ciego con la potencial vacuna contra la lepra *Mycobacterium w* como agente inmunoterapéutico e inmunoprofiláctico contra la lepra, en una región altamente endémica con una tasa de incidencia superior a 18 por 1000 habitantes. Hasta el 31 de agosto de 1992 se evaluaron 224 aldeas con una población de 307.981 habitantes (censo de 1981). Se registró un total de 979 pacientes MB y 2801 pacientes PB. Se examinó un total de 19.453 contactos cotidianos de pacientes de lepra en busca de señales clínicas de la enfermedad, de los cuales, 16.519 recibieron la dosis inicial, mientras que 10.454 también recibieron la dosis de refuerzo de vacuna/placebo. En este artículo se detallan las metas y objetivos del ensayo, su diseño y estado actual, al igual que los aspectos socio-culturales relacionados.

Lepr Rev (1993) **64,** 312–315

Loss of viability of *Mycobacterium leprae* isolated from nasal secretions of lepromatous leprosy patients following daily rifampicin and DDS therapy

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Summary Excreta from blowing their noses was collected from 4 previously untreated multibacillary (LL) patients in the ALERT hospital, immediately before and during daily treatment with 600 mg rifampicin and 100 mg dapsone (DDS). The *Mycobacterium leprae* recovered from the nasal secretions were enumerated and inoculated into the footpads of normal mice. Bacilli recovered from 2 of the patients failed to infect mice after 1 day's treatment, and all infectivity of the bacilli from the other 2 patients was lost after 2 days' treatment. These findings demonstrate the rapidity with which rifampicin-containing multidrug treatment is likely to reduce a patient's level of infection to their contacts.

Introduction

It is generally agreed that the nose is the main exit route of leprosy bacilli from the body.[1–4] Therefore, the quicker *M. leprae* shed from the nose lose their viability because of chemotherapy, the quicker multibacillary patients become non-infectious to their contacts. Previous studies have demonstrated that daily treatment with 600 mg rifampicin results in extremely rapid killing of leprosy bacilli recovered from skin biopsies of multibacillary patients.[5–6] Loss of infectivity for normal mice occurred within 3–7 days, the time the first post-treatment biopsies were taken, indicating that at least 99% of the initial population of viable *M. leprae* had been killed. Similar results were achieved with single doses of 1200 mg rifampicin, while single doses of 900 and 600 mg rifampicin were only slightly less bactericidal.[7] Because there have been no parallel studies to determine how quickly *M. leprae* that have been excreted from the nose are killed by rifampicin-containing treatment, we studied the length of time taken for leprosy bacilli, recovered after the subjects had blown their noses, to lose their infectivity to normal mice.

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Table 1. Loss of infectivity of *M. leprae* isolated from nasal secretions of lepromatous leprosy patients

Duration of treatment (days)	Patient number			
	1	2	3	4
0	8/8	8/8	8/8	8/8
1	0/8	8/8	8/8	0/8
2, 3, 8, 15	0/8	0/8	0/8	0/8

Results are expressed as number of mice showing multiplication/number of mice inoculated with 10^4 AFB (8/8 or 0/8).

Materials and methods

We selected 4 newly-diagnosed and previously untreated multibacillary patients for the study and in 1991 they were admitted to the All Africa Leprosy and Rehabilitation Training Centre (ALERT) hospital for an initial period of daily treatment with 600 mg rifampicin and 100 mg dapsone. Nasal secretions were collected immediately before dose 1 of rifampicin (day 0) and 24 hours after doses 1, 2, 3, 5, 8 and 15 (days 1, 2, 3, 5, 8 and 15) by patients blowing their noses into Petri dishes. The nasal collections were decontaminated by treating with 0·5 N sodium hydroxide for 20 minutes and washed twice with phosphate-buffered saline (PBS) pH 7·2 at a room temperature of 21°C. The leprosy bacilli were then pelleted by centrifugation and resuspended in 2 ml PBS containing 0·1% bovine serum albumin (BSA). The numbers of *M. leprae* present in each nasal excretion were determined by acid-fast staining and 8 BALB/c mice inoculated with 10^4 *M. leprae* in both hind foot-pads.[8] The mice were killed 6 months later, the infected foot-pads washed with alcohol, minced and then homogenized in PBS containing 0·1% BSA until a homogeneous suspension was obtained. The number of bacteria in the homogenates were then determined after acid-fast staining. Multiplication of *M. leprae* was considered to have occurred when more than 10^5 acid-fast bacilli were recovered per foot-pad.

Results and Discussion

The number of *M. leprae* recovered from each nasal excretion of the 4 patients fell from an average of $2{\cdot}7 \times 10^6$ per ml from days 0–3 to about $0{\cdot}6 \times 10^6$ from days 5–15. While the numbers of bacilli shed from each nose fell relatively slowly, the daily treatment with rifampicin and DDS caused a large fall in bacilli viability (Table 1)—i.e. as expected, bacilli from the 4 patients were infectious for mice pretreatment, but 24 hours after dose 1 leprosy bacilli recovered from 2 patients were no longer infectious and 24 hours after dose 2 and on all subsequent occasions all mouse foot-pad infectivity was lost. Because of the incubation with NaoH, and because *M. leprae* were collected only 6 months after the mice had been inoculated, the proportions of viable organisms may have been underestimated to a small degree.

These findings are thus very similar to those previously obtained with bacilli recovered

from skin biopsies[5–7] and suggest that rifampicin-containing treatment is as effective in killing bacilli in nasal secretions as those harboured in skin lesions. They therefore indicate that the level of infection of multibacillary patients for their contacts will be rapidly reduced once chemotherapy has been initiated with the WHO-recommended multidrug treatment.[9] Thus the world-wide implementation of such treatment, which has already resulted in the cure and discharge of over 3 million patients and a substantial reduction in the prevalence of the disease,[10,11] may also make a significant contribution to controlling its transmission.[12,13]

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Perte de viabilité du Mycobacterium leprae isolé des sécrétions nasales de lépreux lépromateux suite à un traitement quotidien de rifampicine et de DDS

H. S. Habte-Mariam et M. Guebre-Xabier

Résumé Nous avons recueilli des sécrétions nasales de quatre sujets multibacillaires (LL) précédemment non traités à l'hôpital ALERT, immédiatement avant et pendant le traitement quotidien consistant en 600 mg de rifampicine et 100 mg de dapsone (DDS). Le *Mycobacterium leprae* recueilli des sécrétions nasales a été dénombré et inoculé dans la semelle de souris normales. Les bacilles recueillis auprès de 2 sujets, n'ont pas réussi à infecter les souris suite au premier jour de traitement tandis que les bacilles provenant des 2 autres sujets avaient perdu leur infectiosité après deux jours de traitement. Ces résultats démontrent la rapidité avec laquelle un traitement médicamenteux combiné content de la rifampicine, est susceptible de réduire l'infectiosité des sujets pour les personnes qui les entourent.

Pérdida de viabilidad de los *Mycobacterium leprae* aislados de secreciones nasales de pacientes de lepra lepromatosa luego de terapia diaria con rifampicina y dapsona

H. S. Habte-Mariam y M. Guebre-Xabier

Resumen En el hospital ALERT se recogieron secreciones nasales de cuatro pacientes multibacilares (LL) no previamente tratados, inmediatamente antes y durante el tratamiento con 600 mg de rifampicina y 100 mg de dapsona. Los *Mycobacterium leprae* recuperados de las secreciones nasales fueron enumerados e inoculados en las plantas de las patas de ratones normales. Los bacilos recuperados de 2 de los pacientes no infectaron a los ratones luego del primer día de tratamiento, y el carácter infeccioso de los bacilos de los otros 2 pacientes se perdió luego de 2 días de tratamiento. Estos hallazgos demuestran la rapidez con la cual un tratamiento multidroga que contenga rifampicina puede llegar a reducir la capacidad infecciosa de los pacientes respecto de sus contactos.

Lepr Rev (1993) **64,** 316–324

Transport of amino-acids across renal brush border membrane vesicles in *Mycobacterium leprae* infected Swiss albino mice—effect of Convit vaccine

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Summary Brush border membrane vesicles prepared from kidneys of *Mycobacterium leprae* infected (non-vaccinated) and vaccinated-infected Swiss albino mice were used to assess the effect of Convit's combined vaccine (BCG + *M. leprae*) on amino acid transport activity across the tubular basement membrane. The protective effect of Convit's vaccine was more pronounced with respect to the uptake of L-alanine than L-aspartate. Uptake of L-lysine showed no significant difference in the different groups. Footpad counts followed characteristic growth curves in the non-vaccinated infected group but showed a lag in the development of peak levels in the vaccinated group. Further Convit's vaccine appeared to have a protective effect on renal impairment in the mouse model of leprosy in the initial stages of infection only, as indicated by the transient reversal of amino acid uptake and a diminution in the footpad counts induced by *M. leprae* infection. No significant ($P > 0{\cdot}05$) protective effect of the vaccine was found in the advanced disease state.

Introduction

Renal involvement associated with leprosy has often been observed in both humans and animals.[1,2] The disease has been shown to affect the glomerulus as well as the tubules,[3,4] both at the structural and functional levels. Tubular damage resulting from the shedding of the epithelial membrane, and consequently the brush border enzymes, has been demonstrated in human cases of lepromatous leprosy.[5] Since this occurs before any histopathological damage, these biochemical parameters of renal tubular function can serve as early markers of renal damage. However, the direct involvement of renal parenchyma by *Mycobacterium leprae* has still not been shown.

It has been demonstrated that both in field trials and in experimental animals Convit's vaccine and other mycobacteria-based vaccines achieve a quick recovery through the

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augmentation of cell-mediated immune responses.[6] In an earlier study, uptake of amino acids by vesicle preparations from *M. leprae*-infected mice has been shown to be only transient, occurring predominantly during the initial stages of infection.[7] In the present study, mice were immunized with Convit's vaccine before *M. leprae* challenge to discover if the transport of nutrients across brush border membrane vesicles (BBMV) could be reversed by vaccination. As far as we know, this is the first study to explore the role of Convit's vaccine in modulation of renal brush border membrane function in experimental leprosy using the uptake of different amino acids across BBMV preparations.

Materials and methods

We used outbred Lacca strain of Swiss albino mice, raised in the animal house of the Postgraduate Institute of Medical Education and Research, Chandigarh, India, for the study. The bacterial counts of *M. leprae*, isolated from skin biopsies of lepromatous leprosy patients (BI 4+ to 6+, MI > 1%), were adjusted to a concentration of 1×10^4 bacilli/mouse footpad for infecting mice.

VACCINE PREPARATION

The vaccine preparation was a mixture of live BCG (obtained from BCG Vaccine Laboratory, Guindy, Madras, India) and killed armadillo-derived *M. leprae* (obtained through the courtesy of Dr R. J. W. Rees, from the Immunology of Leprosy, IMMLEP Bank) at a concentration of 1×10^5 AFB and 3×10^6 AFB per mouse,[8,9] respectively.

IMMUNIZATION OF MICE

Mice were immunized with 30 μl vaccine given subcutaneously in the left hind footpad. After 21 days the animals were injected in the left hind footpad with the same freshly-prepared vaccine, which was diluted $\times 10$ to serve as the booster dose.[10] After 7 days the mice were infected with *M. leprae* in the right hind footpad, subcutaneously. The animals were thus challenged with *M. leprae* 4 weeks after the primary vaccination. This comprised the vaccinated infected group (V-I group). Mice comprising the control group were neither infected nor vaccinated in both the hind footpads. Control animals received 30 μl of normal saline.

DETECTION OF LEPROSY

M. leprae infection was calculated by the amount of bacteria harvested from the infected footpads. The bacteria were stained by the Ziehl–Nelson method and were counted by the modified pin-head method[11] between 1 and 9 months after the infection.

EXPERIMENTAL DESIGN

A total of 150 Swiss albino mice were divided into 3 groups of 50 animals each:

(i) normal controls;
(ii) infected-nonvaccinated (I-NV);
(iii) vaccinated-infected (V-I).

HOW AND WHEN THE ANIMALS WERE KILLED

At least 15 mice, 5 each from the control, I-NV and V-I groups, were killed 1, 3, 6 and 9 months after infection. The mice were lightly anaesthetized with ether and the skin overlying the abdominal surface was wiped clean with spirit. The animals were then killed by cardiac puncture, after the thoracic cavity was cut open with a pair of fine scissors. Both kidneys were removed and used for the preparation of brush border membrane vesicles (BBMV).

PREPARATION OF BRUSH BORDER MEMBRANE VESICLES (BBMV)

Brush border membrane vesicle (BBMV) preparation from the renal cortex was carried out by the method of Malathi *et al.*[12] and the quality of the vesicles was checked as described by Turner & Moran.[13] To summarize, the renal cortex was removed from both the kidneys, suspended in a homogenizing buffer (pH = 7·0) and homogenized for 10 min, using hotline mixer. The resulting homogenate was left for 10 min with $CaCl_2$ on ice and then centrifuged at 6000 g for 15 min. The supernatant was then centrifuged at 43,000 g for 20 min and then the pellet was washed twice using homogenizing buffer. The pellet was then reconstituted in reconstitution buffer (pH = 7·5) and repeatedly passed through a 25-gauge needle. Finally, the vesicle preparation was incubated at 30°C for 15–20 min. This ensured that the membranes were obtained in a vesicular form that was ideally suited for transport studies, and that the vesicles were intact.

The purity of BBMV preparation from the 3 groups of mice killed at different periods was checked by estimating the characteristic renal brush border marker enzymes.[7] Fold enrichment was found to be ranging from 8 to 11% for γ-glutamyl transpeptidase, 12–17% for leucine aminopeptidase and 15–17% for alkaline phosphatase.

TRANSPORT OF AMINO ACIDS

Uptake of L-lysine, L-aspartate and L-alanine was determined by the Millipore filtration technique of Hopfer *et al.*[14] To summarize, 10 μl of BBMV (60–100 μg protein) were incubated at 20°C in the incubation medium that was composed of 50 μM L-aspartate, L-lysine and L-alanine, respectively, and 0·1 μCi of the respective ^{14}C-labelled substrate. The reaction was stopped after 30 seconds by the addition of 5 ml stopping buffer (150 μM NaCl, 1 mM Tris Hepes; pH 7·5). The mixture was filtered through a 0·45 μM Millipore filter. The filter was air dried and radioactivity was counted in a LKB-1215 Rackbeta Liquid Scintillation Counter.

FOOTPAD COUNTS OF *M. LEPRAE*

Each time the mice were killed both the hind footpads of the I-NV group and the right hind footpad of V-I group were counted for *M. leprae* bacteria by the pin-head method.[11]

STATISTICAL ANALYSIS

The Student's *t*-test was used to compare various groups and the values were expressed as mean ± S.E.

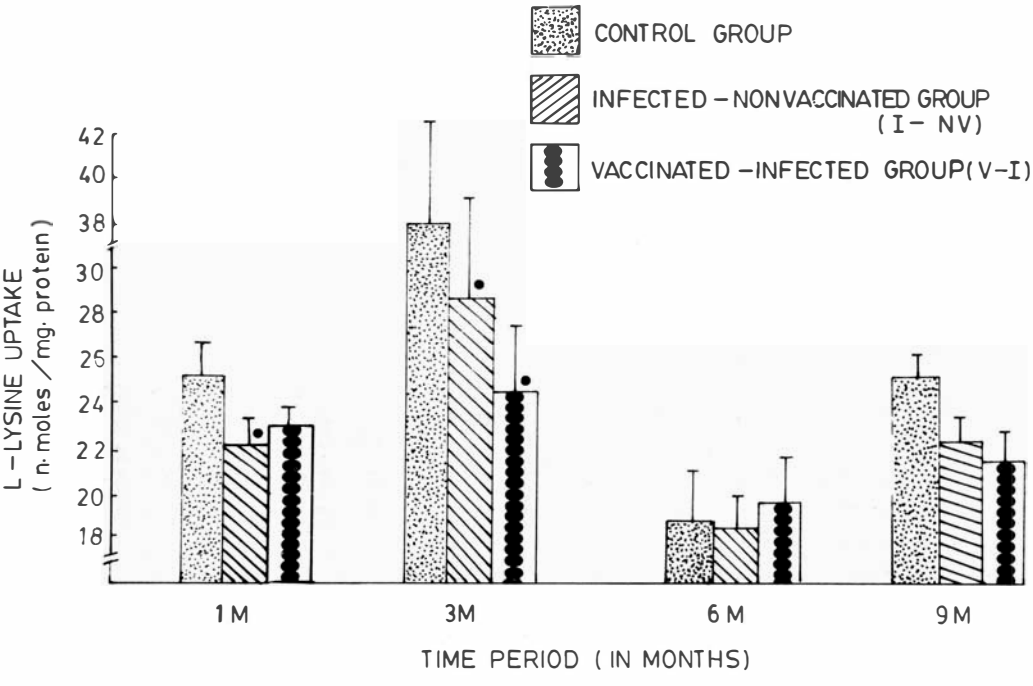

Figure 1. Uptake of L-lysine across renal BBMV in the control, infected and vaccinated groups at 1, 3, 6 and 9 months. The level of significance is: (●) $P < 0{\cdot}001$ *vs.* control group.

Results

Uptake of L-lysine, L-alanine and L-aspartate was determined in renal BBMV prepared from the control, infected and vaccinated-infected groups. Any differences in the uptake of different amino acids in the animals infected with *M. leprae* compared to controls would provide good evidence of an altered biochemical status of the renal tubules in response to *M. leprae* infection.[7]

Uptake of L-lysine across BBMV was decreased significantly ($P < 0{\cdot}001$) at 1 and 3 months post-infection in the I-NV group compared to the control group, but there was no significant difference at 6 and 9 months post-infection (Figure 1), which demonstrates the transient nature of the altered uptake of nutrients. The vaccinated group (V-I), on the other hand, showed no significant difference ($P > 0{\cdot}05$) in the uptake of L-lysine when compared to I-NV group at different periods (Figure 1). Similarly no significant difference ($P > 0{\cdot}05$) was observed in the uptake of L-aspartate across BBMV between the I-NV and V-I groups compared to the control group at different periods of study between 1 and 9 months (Figure 3). Increase in uptake of L-aspartate was observed in the V-I group between 1 and 9 months as compared to its corresponding I-NV group and between 1 and 6 months as compared to its corresponding control group. However, the increase observed in the uptake of L-aspartate was statistically insignificant.

Uptake of L-alanine across BBMV showed a significant difference ($P < 0{\cdot}001$) at 3 and 6 months post-infection between the I-NV group compared to the control group (Figure 2), although at 1 month post-infection no significant difference was observed between the

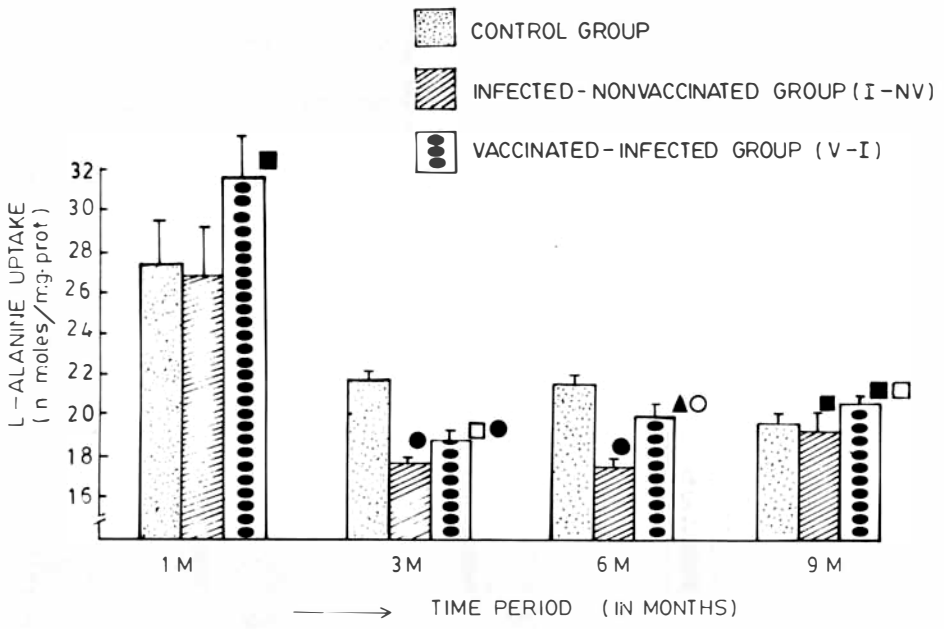

Figure 2. Uptake of L-alanine across renal BBMV in the control, infected and vaccinated groups at 1, 3, 6 and 9 months. The level of significance is: (●) $P<0{\cdot}001$ *vs.* control group; (○) ($P<0{\cdot}01$) *vs.* control group; (■) ($P<0{\cdot}05$) *vs.* control group; (□) ($P<0{\cdot}05$) *vs.* infected group; (▲) ($P<0{\cdot}001$) *vs.* infected group.

2 groups. The V-I group, on the other hand, showed a significant increase ($P<0{\cdot}05$) in the uptake of L-alanine across BBMV between 1 and 6 months post-infection, although the increase was more pronounced at 6 months post-infection ($P<0{\cdot}001$) when compared to the corresponding I-NV group. Increase in the uptake of L-alanine in V-I group compared to I-NV group, however, failed to achieve the level observed in the control group (Figure 3).

Footpad counts of *M. leprae* at different periods were found to follow the characteristic growth curve in both the I-NV and the V-I groups. This was demonstrated by the linear increase in footpad counts between 3 (ranging from $0{\cdot}56\times10^4$ AFB/ml to $0{\cdot}846\times10^4$ AFB/ml) and 6 months post-infection (ranging from $0{\cdot}98\times10^5$ AFB/ml to $1{\cdot}64\times10^5$ AFB/ml), which reached a stationary phase at 9 months post-infection with a mean count of $1{\cdot}39\times10^5$ AFB/ml (Table 1). The V-I group, on the other hand, showed a decrease in the footpad bacillary counts at different periods compared to I-NV group, though the decrease was statistically insignificant ($P>0{\cdot}05$) (Table 2). The bacterial counts in the V-I group showed a characteristic growth curve with a linear increase in the bacillary count between 3 and 6 months and reaching stationary phase at 9 months. Lag in the development of peak counts was observed in the V-I group as compared to the I-NV group.

Discussion

We carried out studies on the uptake of L-lysine, L-aspartate and L-alanine to assess the

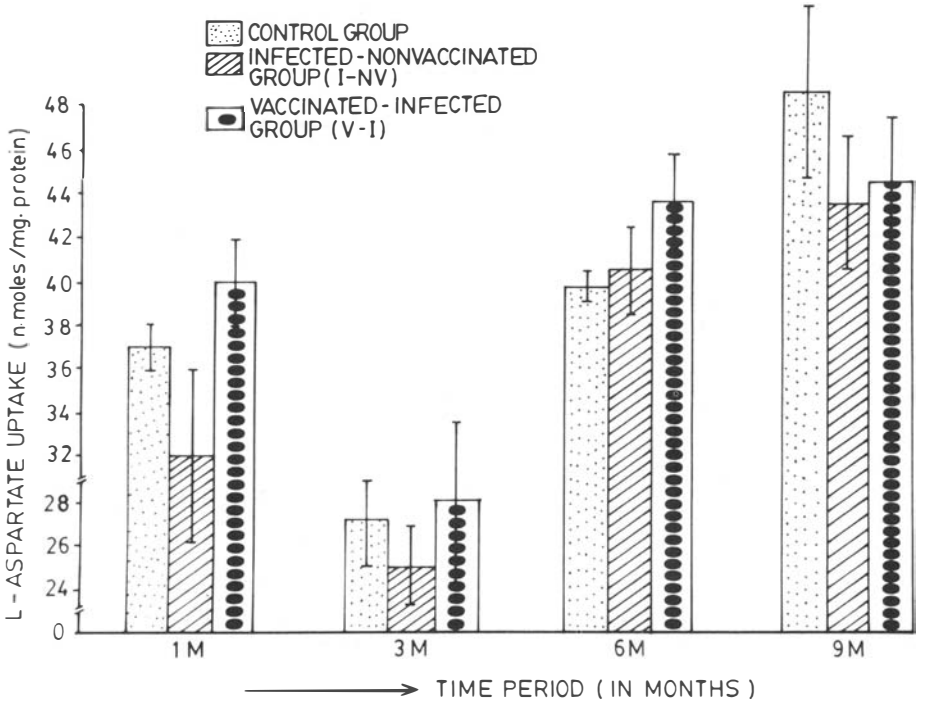

Figure 3. Uptake of L-aspartate across renal BBMV in the control, infected and vaccinated groups at 1, 3, 6 and 9 months.

biochemical functional status of the renal tubule at the brush border membrane level and to evaluate the effect of Convit's combined vaccine on leprosy in mice.

There was a transient alteration in the uptake of L-lysine, L-alanine and L-aspartate across BBMV between 3 and 6 months post-infection, which then returned to normal. These findings are in agreement with our previous observations on leprosy in mice[7] and pyelonephritis in rats.[15] The difference in the tubular uptake of different amino acids could be due to different carrier systems for different amino acids,[16] or multiple carrier systems in renal brush border membrane for handling the organic cation,[17] or to significant internephron heterogeneity for amino acid reabsorption.[18]

In the present study, the protective effect of the combined vaccine has been demonstrated by the increased uptake of L-aspartate (1–3 months post-infection) and L-alanine (1–6 months post-infection), thereby reflecting an improvement in the altered reabsorption capacity of tubular segments. However, no protective effect of vaccine could be observed in the uptake of L-lysine. Variable response of vaccine to different amino acids is possibly due to differences in the carrier systems for individual amino acids.[16] The present study demonstrated only limited arrest of infection by vaccination, as is shown by the increase in the lag phase and low bacterial footpad counts during the early phase of infection. It is during this period that the increased uptake of amino acids was observed in the vaccinated group (V-I) compared to the infected-nonvaccinated group. This finding is

Table 1. Bacterial footpad counts (I–NV group)

Month period	Counts of AFB	
	Range of AFB/ml	Mean ± S.E. (AFB/ml)
1	—	—
3	$0{\cdot}516 \times 10^4 - 0{\cdot}84 \times 10^4$	$0{\cdot}696 \times 10^4 \pm 0{\cdot}120 \times 10^4$
6	$0{\cdot}98 \times 10^5 - 1{\cdot}64 \times 10^5$	$1{\cdot}30 \times 10^5 \pm 0{\cdot}34 \times 10^5$
9	$1{\cdot}14 \times 10^5 - 1{\cdot}64 \times 10^5$	$1{\cdot}39 \times 10^5 \pm 0{\cdot}25 \times 10^5$

Table 2. Bacterial footpad counts (V–I group)

Month period	Counts of AFB	
	Range of AFB/ml	Mean ± S.E.
1	—	—
3	$0{\cdot}525 \times 10^4 - 0{\cdot}810 \times 10^4$	$0{\cdot}690 \times 10^4 \pm 0{\cdot}10 \times 10^4$
6	$0{\cdot}710 \times 10^5 - 1{\cdot}30 \times 10^5$	$1{\cdot}10 \times 10^5 \pm 0{\cdot}120 \times 10^5$
9	$0{\cdot}735 \times 10^5 - 1{\cdot}35 \times 10^5$	$1{\cdot}17 \times 10^5 \pm 0{\cdot}51 \times 10^5$

consistent with the protective effect of immunization by *M. leprae*[6] which is evident by a significant fall in footpad counts at 6 and 9 months. Our findings showed a consistent lag in bacterial footpad counts in reaching the plateau level after 6 months. The differences between the individual series of assays on the uptake of amino acids at different times could be due to certain differences in the bacterial load of micro-organisms in individual animals in a particular group that could have occurred during the course of infection. Since the animals were monitored at 1, 3, 6 and 9 months only, in-between fluctuations could have occurred which might lead to differences in individual series of assays on the uptake of amino acids.

Reversal of the transient alterations in the uptake of different amino acids seen in BBMV preparation in the vaccinated group during the early post-infection phase may have implications, viz: that biochemical changes across the renal brush border membrane were specific to *M. leprae* infection, as vaccination tends to revert the values to normal. In addition, reversal of amino-acid uptake in the vaccinated group could be related to a lag in the development of peak bacterial load and lower counts during the 3–6 month period corresponding to the infected-nonvaccinated group (I-NV). It is possible that the Convit's vaccine prevents the formation of immune-complexes by disturbing the ratio of antigen : antibody required for the formation of immune complexes. Based on these observations, it is reasonable to suggest that Convit's combined vaccine does play a role in improving the functional status of the renal brush border with respect to the amino-acid uptake, though vaccination alone fails to limit the disease. Furthermore, this study has shown that amino-acid uptake could be used as a physiological marker in experimental models to assess the effect of vaccination, and that antileprosy drugs, in conjunction with vaccination, could limit infection successfully.

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Lepr Rev (1993) **64,** 316–324

Transport des amino-acides à travers les vésicules rénales de la membrane à bordure en brosse chez des souris Swiss Albino infectées par le Mycobacterium leprae—Effet du vaccin de Convit

MANJULA KOHLI, SURINDER KAUR, NIRMAL K. GANGULY, VINOD K. SHARMA ET KIRPAL S. CHUGH

Résumé Nous avons utlisé des vésicules de la membrane à bordure en brosse préparées à partir des reins de souris Swiss Albino infectées par le *Mycobacterium leprae* mais non vaccinées et infectées et vaccinées, pour évaluer l'effet du vaccin combiné de Convit (BCG + *M. leprae*) sur l'activité de transport des amino-acides à travers la couche sous-épithéliale tubulaire. L'effet protecteur du vaccin de Convit était plus prononcé dans l'absorption de L-alanine que dans celle de L-aspartate. L'absorption de L-lysine n'a présenté aucune différence significative entre les divers groupes. Les comptes plantaires suivaient des courbes de croissance caractéristiques dans le groupe infecté non vacciné mais présentaient un retard dans le développement des concentrations-pics chez le groupe vacciné. Il semblerait d'autre part, que le vaccin de Convit n'a un effet protecteur sur le délabrement rénal chez la souris dans la lèpre, qu'aux premiers stades de l'infection comme indiqué par l'inversion passagère de l'absorption d'amino-acides et par une diminution des comptes plantaires induites par le *M. leprae*. Dans la phase avancée de la maladie, l'effet protecteur du vaccin ne s'est pas révélé significatif ($P > 0{,}05$).

Trasporte de aminoácidos a través de las vesículas de la membrana renal de borde piloso en ratones albino suizo—efecto de la vacuna Convit

MANJULA KOHLI, SURINDER KAUR, NIRMAL K. GANGULY, VINOD K. SHARMA Y KIRPAL S. CHUGH

Resumen Se utilizaron vesículas de la membrana de borde piloso tomadas de rinones de ratones albino suizo infectados con *Mycobacterium leprae* (no vacunados) y ratones infectados mediante vacuna, para evaluar el efecto de la vacuna combinada Convit (BCG + *M. leprae*) sobre la actividad de traspaso de aminoácidos a través de la membrana de base tubular. El efecto protector de la vacuna Convit fue más pronunciado en lo que se refiere al consumo de L-alanina y L-aspartato. El consumo de L-lisina no presentó diferencias significativas en los diversos grupos. Los recuentos obtenidos de las patas siguieron las curvas de aumento características en el grupo infectado no vacunado, pero observaron un retraso en el desarrollo de niveles pico en el grupo vacunado. Más aún, la vacuna Convit sólo parece tener un effecto protector sobre las deficiencias renales en el modelo de lepra de los ratones durante las etapas iniciales de infección, tal como lo indica la inversión transitoria del consumo de aminoácidos y la disminución en los recuentos de patas inducidos mediante la infección de *M. leprae*. La vacuna no demostró un efecto protector significativo ($P > 0{,}05$) en el estado avanzado de la enfermedad.

Lepr Rev (1993) **64,** 325–329

Hospital-based epidemiological study of reactions, Buluba Hospital, 1985–89

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Summary A retrospective study of 256 reactional episodes, both reversal reaction and erythema nodosum leprosum (ENL), seen in Buluba Hospital over a 5-year period (1985–89) was made. Over 90% of these episodes were due to reversal reaction, with ENL being encountered infrequently. About 80% of reversal reactions occurred during chemotherapy but all the episodes of ENL occurred during this period. Over 70% of both reversal and ENL episodes presented with clinically apparent nerve and skin involvement.

The need to assess the effect of multidrug therapy on the incidence of reactions and to develop more sensitive diagnostic tools to detect early neuritis is emphasized. It is also necessary to study those patients who develop recurrent reactional episodes.

Introduction

Reactions are a common phenomenon among leprosy patients and the early detection and proper management of reactional episodes is an important part of the success of any leprosy control programme.[1] Unfortunately there is little information on the occurrence and distribution of these episodes in Uganda.

Buluba Hospital offers specialized services for the treatment of complications of leprosy including reactions. This study was carried out to establish some epidemiological characteristics of reactions and to provide a data base for future prospective studies on reactions.

Materials and methods

The case notes of all the patients admitted for reactions (reversal and erythema nodosum leprosum) over a 5-year period (1985–89) were retrieved from the hospital records office and analysed for: the type of leprosy, the type of reaction, age, sex, the timing of reactions, the type of chemotherapy, the pattern of reactions, and the frequency of reactional episodes. The hospital's annual reports for the same 5-year period were also consulted to obtain information about leprosy patients registered for chemotherapy in its catchment area.

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Table 1. Distribution of admissions by leprosy type

Leprosy type	Number of patients for Reversal reaction	ENL	Total
BT	130	0	130
BB	26	0	26
BL	32	9	41
LL	0	9	9
Total	188	18	206

Results

GENERAL TRENDS

There were 2317 leprosy admissions during the 5-year study period and 206 of these were admitted for reaction. Some of the patients were admitted more than once, giving a total number of 256 admissions for the management of acute episodes—231 were for reversal reaction (RR) and 25 for erythema nodosum leprosum (ENL) reaction. Of 188 patients admitted for reversal reactions, 130 (69·1%) were classified as borderline tuberculoid. Only 18 patients presented with ENL (Table 1).

The combined total of registered patients for the 5-year period was 11,688—2743 (23·5%) belonged to the multibacillary type (BB, BL, LL), 4643 (39·7%) were the borderline tuberculoid type (BT) and 4302 (36·8%) were the TT type.

REACTIONS AND AGE

Age was known for 117 (56·8%) of the patients. The remaining 89 (43·2%) were simply recorded as 'adults'.

REACTIONS AND SEX

Of the 206 patients studied, 116 were males and 90 were females, a male to female ratio of 1·3:1. Among those with reversal reaction, 103 were males and 85 were females, a male to female ratio of 1·2:1. Of the 18 patients with ENL, 13 were males and 5 were females, a male to female ratio of 2·6:1.

TIMING OF REACTIONS

This was taken as the period in which reactions occurred relative to chemotherapy—that is, before, during or after chemotherapy—206 (80·5%) episodes occurred during chemotherapy, 38 (14·8%) before and 12 (4·7%) after chemotherapy. Of the 231 RR episodes, 181 (78·3%) occurred during chemotherapy, and all the 25 ENL episodes occurred during this period in 9 borderline lepromatous (BL) and 9 lepromatous leprosy (LL) patients. The RR episodes during chemotherapy occurred in 104 borderline tuberculoid (BT), 23 mid-borderline (BB) and 13 BL patients. The RR episodes before

chemotherapy occurred in 38 patients, 23 of whom were classified as BT, 3 as BB and 12 as BL. The RR episodes after chemotherapy occurred in 10 patients; 3 BT and 7 BL. In this group all the patients, with the exception of 2 BT patients, had received multiple drug therapy. In 2 BT patients (including the patient with MDT), RR commenced within 6 months of stopping chemotherapy. In 6 BL patients and the single BT patient, reactions developed between 6 and 12 months after stopping chemotherapy. In the remaining BL patient, the reaction occurred 2 years after stopping chemotherapy.

CHEMOTHERAPY

Of the 206 patients, 134 (65%) were on multiple drug therapy (MDT), according to World Health Organization recommendations.[2] For multibacillary patients who had positive skin smears after 2 years' treatment, MDT was continued up to smear negativity. In all, 117 (62·2%) patients with RR and 17 of 18 patients with ENL were on MDT. The remaining 72 patients were on alternative regimens, including Dapsone monotherapy.

PATTERN OF REACTIONS

Of the 231 reversal episodes, 168 (72·7%) had involvement of both skin and nerves. Of the 25 ENL episodes, 18 (72%) had both skin and nerves involved and 7 (28%) had skin involvement alone.

FREQUENCY OF EPISODES

In total, 151 (80·3%) patients with RR suffered only 1 episode of reaction; 31 (16·5%) 2 episodes and 6 (3·2%) 3 episodes; 11 of the 18 patients with ENL had 1 episode and 7 had 2 episodes.

Discussion

It is estimated that by 1987 there were 10,613 registered leprosy patients in Uganda.[3] In this same year 2094 patients were in the Buluba Leprosy Control area. Some of these patients risk developing reactions, and without prompt and proper treatment, disability and deformity can easily follow from nerve damage.

Little or no progress has been made in research on leprosy reactions and nerve damage, 2 important areas of prevention and treatment.[4] Only a few studies based on population and community surveys on reactions and their epidemiology have been conducted, and, certainly, not in Uganda.

Reactions are the third commonest cause for admissions of leprosy patients to Buluba Hospital.[5] Over the study period they accounted for 11% of leprosy admissions. The fact that the 256 admissions were taken out of a cumulative total of 11,688 patients suggests that reactions requiring hospital management arise in about 2·2% of all registered patients. This figure is further confirmed because Buluba Leprosy Centre is the only facility in the area where patients with severe reactions are likely to be referred to or present themselves voluntarily. The patients in the catchment area are routinely visited by field workers. Boerrigter[6] observed that 2·2% of patients developed marked reactions during MDT.

The commonest type seen was RR, which accounted for 90·2% of reaction admissions. In this study ENL represented 9·8% of the total reactions, a similar result to that reported by Becx-Bleumink[7] in a study carried out in Ethiopia.

Because the ages of only 43·2% of the patients were known, no realistic conclusions on age applicable to the whole group could be made.

There was a slight preponderance of males among those who developed reactions. Studies elsewhere have shown males to be more frequently affected up to a ratio of 6:1.[8] There is a slight male preponderance, with respect to RR alone, while in the group who developed ENL, the male to female ratio was 2·6:1. The latter observation may be related to the fact that there are generally fewer females with BL and LL leprosy.[9]

Most of the reactions (80·5%) occurred during chemotherapy, with all ENL episodes occurring during this period. A total of 65% were on MDT. The MDT coverage in this area during this period rose from about 6% in 1986 to about 69% in 1989.[10] A more specific study is needed to establish if MDT is related to the frequency of reactional episodes. The observation that 4·6% of reactions presented after completion of chemotherapy could be used to determine whether post-treatment surveillance is cost-effective or not, but it would have to be calculated against the size of the population at risk and preferably for each leprosy type, as was the case in the study of Becx-Bleumink.[7]

The Ugandan reaction pattern has already been described by Blenska,[11] but without quantitative data. In our study, 72·7% of reversal episodes presented with symptoms and signs involving skin and nerves while 13 out of 18 ENL reactions involved both skin and nerves. That none of the patients had neuritis alone without skin manifestations could be due to lack of specific diagnostic tools in Buluba's Leprosy Control Programme.

It is noteworthy that 19·7% of all RR patients and 7 out of 18 ENL patients experienced more than one episode. Recurrent reactional episodes are associated with a great risk of developing permanent nerve damage. This group of patients should be studied to determine why they are prone to such recurrent reactional episodes.

Acknowledgement

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Lepr Rev (1993) **64,** 325–329

Etude épidémiologique des réactions conduite en milieu hospitalier, Hôpital Buluba, 1985–1989

R. Bwire et H. J. S. Kawuma

Résumé Nous avons fait une étude rétrospective de 256 épisodes réactionnels, tant réactions lépreuses qu'érythème noueux lépreux (ENL), observés à l'Hôpital Buluba sur une période de cinq ans (1985–1989). Plus de 90% de ces épisodes étaient dus à des réactions lépreuses tandis que l'érythème noueux était peu fréquemment observé. 80% environ des réactions lépreuses ont eu lieu pendant la chimiothérapie mais tous les épisodes d'ENL se sont présentés pendant cette période. Plus de 70% des épisodes de réactions lépreuses et d'ENL ont présenté des complications nerveuses et cutanées cliniquement visibles.

Nous soulignons le besoin d'évaluer l'effet d'un traitement médicamenteux combiné sur l'incidence des réactions et de développer des outils de diagnostic plus sensibles pour détecter la névrite précoce. Il y a également lieu d'étudier le cas des sujets qui développent des épisodes réactionnels récurrents.

Estudio epidemiológico de reacciones dentro de un hospital, Hospital Buluba, 1985–1989

R. Bwire y H. J. S. Kawuma

Resumen Se efectuó un estudio retrospectivo de 256 episodios de reacciones, tanto de inversión como de eritema nodosum leprosum (ENL), observados en el Hospital Buluba durante un período de cinco años (1985–89). Más de un 90% de estos episodios se debió a una reacción inversa, siendo muy poca la frecuencia de ENL. Aproximadamente un 80% de las reacciones inversas ocurrieron durante la quimioterapia, mientras que todos los episodios de ENL se produjeron durante este período. Más de un 70% de los episodios de inversión y de ENL se presentaron con participación de nervio y piel clínicamente aparente. Se enfatiza la necesidad de evaluar el efecto de la terapia de drogas múltiples sobre la incidencia de reacciones, y también de dessarrollar instrumentos de diagnóstico con mayor sensibilidad para una temprana detección de la neuritis. También es necesario estudiar a los pacientes que desarrollan episodios reactivos recurrentes.

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Regeneration at the predilective damage sites of nerve trunks in treated leprosy

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Summary Superficially located large and medium sized mixed peripheral limb nerves in active leprosy have previously been shown to have well-recognized fusiform swellings. It is generally agreed that these are the sites of predilective nerve involvement where the severest degeneration and fibrosis occur. A semiquantitative histopathological study on one of these sites, the flexor retinaculum region of the posterior tibial nerve, has been carried out on 14 treated leprosy patients who suffered from total sensory loss to the foot for between 2 and 40 years. The following observations were made: (1) large-scale nerve regeneration was present as characterized by numerous Schwann cells and unmyelinated axons which formed regeneration clusters; (2) thick myelinated axons were either absent or present only in very low numbers; (3) the intraneurial fibrosis was usually not severe; (4) the presence of active inflammation probably interfered with nerve regeneration; (5) it appeared that this regeneration started shortly after the onset of therapy and persisted for decades; (6) lepromatous cases were characterized by evenly distributed pathology, whereas borderline tuberculoid cases had an unevenly distributed pathology; (7) the massive nerve regeneration observed was functionally ineffective—these findings indicate that the total nerve damage may affect the more peripheral nerve branches.

Introduction

Leprosy, with all its variety of skin manifestations, is essentially a peripheral nerve disease. Most previous studies on the histopathology of peripheral nerves in leprosy have

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been confined to cutaneous nerve branches. Those studies on the histopathology of large mixed peripheral nerves in leprosy, all of them dealing with active cases, revealed either no[1,2] or limited nerve regeneration.[3–6] Dastur *et al.* [4] demonstrated that the scanty nerve regeneration observed in active leprosy was blocked by fibrosis at the predilective damage sites of large mixed peripheral nerves of the limbs. These segments of predilective nerve involvement, manifesting themselves as fusiform swellings, are superficially located.[7] Such segments are generally considered to be the sites of the most severe damage and hence fibrosis.[4,8]

To our knowledge, the present report is the first devised to study the site of predilective nerve involvement in treated leprosy cases suffering from loss of sensation. The primary goal of this study was to describe the distinctive histopathological patterns, with special attention to nerve regeneration, in the nerve trunks of advanced lepromatous (LL), borderline lepromatous (BL) and borderline tuberculoid (BT) leprosy cases that had received treatment.

Material and methods

We selected the lower third of the posterior tibial nerve for this study because it was more easily available than other nerve trunks, and in all 14 specimens were studied (Table 1). The nerves in cases 3, 4 and 8–14 were removed from patients undergoing muscle graft reconstruction,[9] and in cases 1, 2 and 5–7 we removed nerves from the amputated legs of leprosy patients immediately following surgery. All patients received anti-leprosy treatment. We collected 4 control samples (20–39 years) from the Department of Forensic Medicine, Menelik Hospital, Addis Ababa. In these cases, autopsy revealed no significant alterations except for the physical injury causing death. The control samples were collected within 8 hours of death.

From both the study and control cases, posterior tibial nerve samples were taken from under the upper half of the flexor retinaculum. All blocks were cut transversely. The following histological methods were employed. For general orientation and collagen: haematoxylin and eosin, and van Gieson. For myelin sheaths: Luxol fast blue staining on Helly fixed samples and 0·5% osmium fixation followed by paraffin processing. For axons: modified Schoefield's silver impregnation on fresh-frozen cryostat sections fixed in 10% neutralized formaldehyde; and avidin-biotin immunoperoxidase method utilizing anti-neurofilament 200 kD primary antibody (Boehringer, Mannheim, Germany) on ethanol-acetic acid-fixed paraffin sections. For Schwann cells: avidin-biotin immunoperoxidase method using anti-S-100 primary antibody (Dakopatts, Glostrup, Denmark) on buffered formaldehyde fixed paraffin sections, with no enzymatic digestion. Although the immunoperoxidase method for axons was more reproducible than the silver impregnation, the latter depicted the finest regenerating axons. For semiquantitative purposes, we found osmication and Luxol fast blue methods equally suitable. The parameters assessed are listed in Table 1.

Results

Although the lower third of the posterior tibial nerve was firm on palpation, no fusiform swelling was detected during surgery or macroscopical investigation of the dissected, amputated legs.

Table 1. Histopathological findings on posterior tibial nerves and clinical characteristics of treated leprosy patients

Case no.	Sex	Age	Class	Post Tx	LOS yrs	No. of reactions	Endo/EPI neural fibrosis	Peri-neural fibrosis	Inflammation			No of Schwann cells	No of axons		No. of myelin sheaths		Distribution of pathology	
									LEPR	TUB	Non-spec		thin	thick	thin	thick	even	uneven
1	M	38	LL	2 mths°	>15	0	+	+	+	−	−	++++	++++	±	+±	−	+++	−
2	M	34	LL	20 mths°	20	2	+	+	+	−	−	++++	++++	±	+±	−	+++	−
3	M	15	LL	—**	3	0	+	+	±	±	±	++++	++++	±	++	−	+++	−
4	F	16	LL	$4\frac{1}{2}$ yrs*	>3	1	+	+±	−	−	±	++++	++++	±	++	−	+++	−
5	M	67	LL	36 yrs°	>40	NA	+	+±	−	−	−	++++	++++	+	++±	±	+++	−
6	M	50	BL	10 yrs°	>10	NA	+	+	+	−	−	++++	++++	+	++	+	++	+
7	M	55	BL	24 yrs°	>25	NA	+±	+	−	−	−	++++	++++	+	++	+	++	+
8	M	21	BL	1 mth*	2	2	+±	+	−	±	−	+++	+++±	+	++	±	+±	+±
9	F	16	BL	14 mths*	>2	0	+±	+	−	−	+	+++	+++±	+	++	+	+±	+±
10	F	18	BL–BT	14 mths*	>3	0	++	+	−	+	+	+++	+++	+	++	±	+	++
11	M	17	BT	$2\frac{1}{2}$ yrs***	3	2	+±	+	−	++	++	++	+	±	±	±	−	+++
12	M	22	BT	6 mths*	4	0	+±	+	−	+±	++	++±	+	±	−	−	−	+++
13	F	16	BT	2 yrs°	>2	0	++	++	−	±	+	++±	+++	+	++	+	−	+++
14	M	25	BT	1 yr°	8	0	+++	++	−	−	+	+	++	±	+	−	−	+++

Abbreviations: Tx = Therapy; LOS = Loss of sensation; NA = information not available; LEPR = Lepromatous; TUB = Tuberculoid.

For semiquantitative evaluation, a grade system was used: − no; + slight/few; ++ moderate/several; +++ marked/many; ++++ above normal; ± intermediate grades. Control nerves were the normal reference value (+++) concerning the number of Schwann cells, axons and myelin sheaths. 'Uneven +++' means that the pathology was non-homogeneously distributed between and within individual fasciculi. 'Even +++' pathology means that the fascicular pathology was homogeneous.

° Time since released from Tx.

* Patients not released from Tx, although the WHO multidrug regime completed. In these cases duration of extra Tx was specified. All were receiving Tx at surgery.

** WHO multidrug Tx not completed. Attendance rate at surgery: 15/24 months.

*** Drugs taken irregularly.

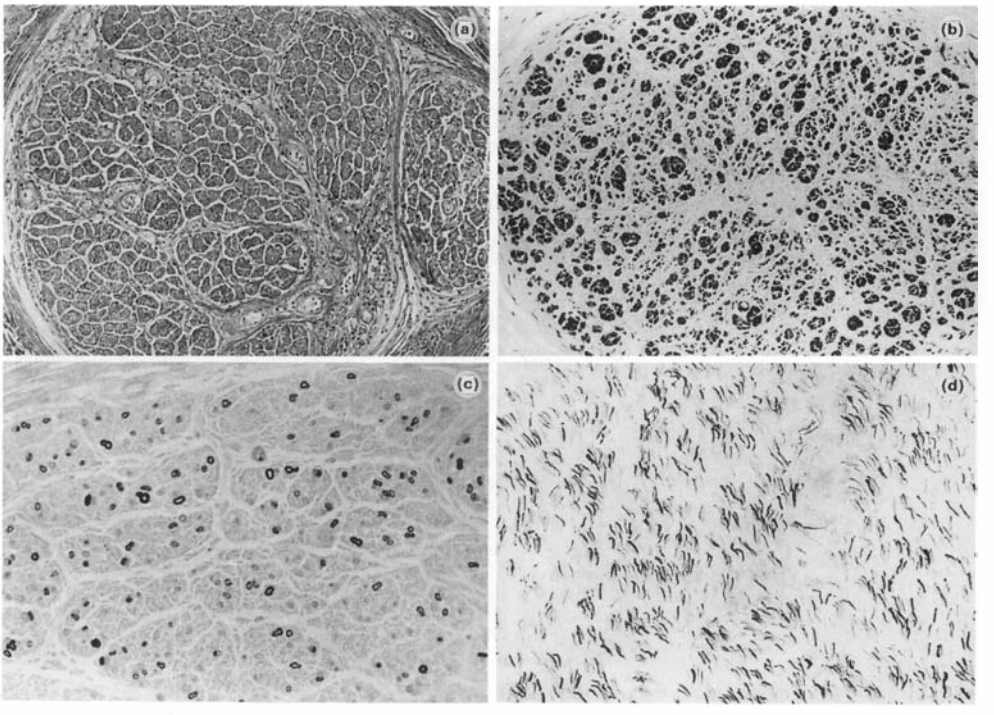

Figure 1. Histology of nerve trunks in lepromatous (LL) leprosy. a. The fascicle consists of evenly-distributed regeneration clusters of similar size. Van Gieson, ×115·5. b. The entire cross-sectional area of this fascicle reveals numerous Schwann cell processes, many arranged in discernible clusters. S-100, avidin-biotin complex, horseradish peroxidase method, ×115·5. c. The myelin pattern is characterized by a moderate number of evenly-distributed, small diameter sheaths. The contours of the Schwann cell clusters are discernible. Osmium fixation, paraffin embedding, ×462. d. The axonal pattern is characterized by a high number of evenly distributed thin axons. Modified Schoefield's method, ×462.

The major clinical and microscopical features are summarized in Table 1. The overall histological pattern of nerve trunks was homogeneous in LL cases (nos 1–5). All fasciculi appeared similar, and consisted of evenly distributed regeneration clusters (Figure 1). In contrast, the pattern in BT (cases 11–14) was not homogeneous. This pattern was characterized by increased and uneven fibrosis, and all pathological changes as well as nerve fibres of varying diameter were distributed in an uneven manner. In addition, focal changes were present within individual fasciculi (Figure 2). Lastly, in BL cases (nos 6–10) the pattern was intermediate with regard to fibrosis, inflammatory infiltrate and the diameter of nerve fibres.

Specifically, the degree of the residual inflammatory infiltrate was usually limited in cases 1–10, 13 and 14. In the case of slight lepromatous infiltrate, small numbers of foamy cells were present. Small epithelioid cell granulomas represented slight tuberculoid infiltrate, and in addition there was non-specific inflammation which consisted of mononuclear cells. In cases 11 and 12, large tuberculoid granulomas were present, surrounded by many lymphocytes (Figure 2b).

In LL and BL cases (nos 1–10) the Schwann cell number was high, usually above normal, coupled with similarly high numbers of unmyelinated axons. The Schwann cell processes formed groups which corresponded to regeneration units (Figure 1a, b). In the same cases, the number of myelinated axons was markedly decreased, so that there was a

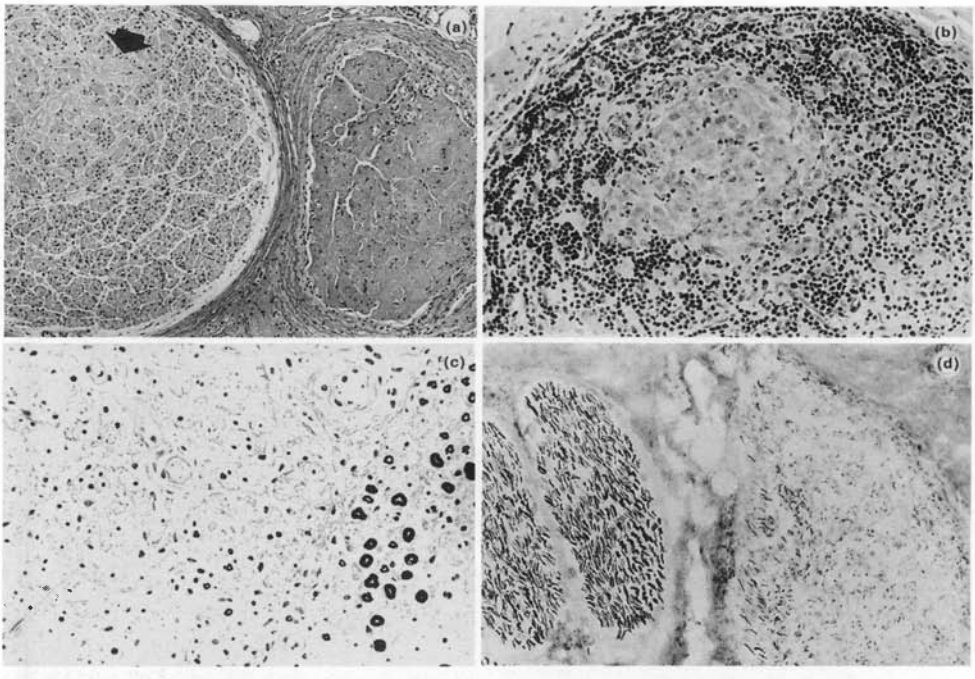

Figure 2. Histology of nerve trunks in borderline tuberculoid (BT) leprosy. a. The fascicle on the right shows advanced fibrosis. The fascicle on the left consists mostly of regeneration clusters; however, focal fibrosis is also present (arrow). H & E, × 115·5. b. The neural tissue in this fascicle has been destroyed by a large tuberculoid granuloma surrounded by numerous mononuclear cells. H & E, × 231. c. This fascicle reveals marked loss of myelin sheaths. Note that the myelin sheaths show a much larger diameter range than those in lepromatous leprosy. Osmium fixation, paraffin embedding, × 462. d. The marked inflammation in the right fascicle resulted in marked loss of axons. The left fascicle reveals numerous axons of varying thickness. Modified Schoefield's method, × 115·5.

strong shift towards small diameter axons. The myelin population was predominated by thin sheaths of small calibre which corresponded to newly-formed myelin surrounding regenerating axons (Figure 1c, d). In cases 1–10, the numbers of both the unmyelinated and the newly-myelinated regenerating axons showed little variation, irrespective of the time elapsed since the onset of therapy. In the presence of tuberculoid infiltrates (cases 11 and 12), the number of Schwann cells was lower than in control cases and the lack of axons and myelin sheaths was conspicuous (Figure 2c, d). The most severe decrease of Schwann cells occurred together with marked fibrosis (case 14)—however, in this case the number of unmyelinated axons was not as low as in cases 11 and 12 which showed tuberculoid inflammation.

Discussion

Autopsy studies on peripheral nerves in active leprosy reveal the presence of spindle-shaped thickenings at the sites of predilection in lepromatous cases, and more uniform thickenings in tuberculoid cases.[1,2] The lack of the fusiform swelling in the ankle region of the posterior tibial nerve in our cases of advanced treated leprosy suggested that this swelling was characteristic of active leprosy. The swelling might have resulted from the

inflammatory infiltrate and from the oedema present during the active phase of leprosy. The perineural fibrosis observed in our treated LL cases, probably for the same reason, was also less conspicuous than that usually reported in untreated leprosy.[10]

The pattern of numerous Schwann cell processes, moderate numbers of thin myelin sheaths and high numbers of thin regenerating axons observed in nerve trunks is typical of active but ineffective and persistent nerve regeneration.[11–13] The few thick axons probably represent surviving fibres in numbers insufficient to provide any useful function. In LL and BL cases there appeared to be a complete lack of correlation between the degree of regeneration and the time elapsed since the start of therapy (see the extreme cases, 3 and 5). This fact suggested that the axonal regeneration may start shortly after the onset of therapy and may persist for decades. Large-scale nerve regeneration might also occur in BT leprosy, provided neither fibrosis nor inflammation were severe (case 13).

There seemed to be 2 exceptions to the above general pattern. First, a significant residual inflammatory infiltrate was present in 2 BT patients who were still receiving treatment (cases 11 and 12). Case 12 had completed the WHO multidrug therapy only 6 months before surgery, whereas in case 11 the drugs had been taken irregularly. As in these cases the number of Schwann cells was still high, these cells either survived the most active phase of inflammation or they regenerated following this event. As axons were almost absent this would indicate that inflammation was hindering axonal regeneration;[13–14] the absence of axons within the epithelioid granulomata also supported this theory. Secondly, case 14 was characterized by advanced fibrosis. This marked fibrosis seemed to result in a decreased number of Schwann cells. Despite the low number of Schwann cells, the number of regenerating axons was higher, in the absence of inflammation, than in cases 11 and 12, where inflammation was present. This also suggested that large-scale nerve regeneration may start only after a significant decrease of inflammation.

No histological change could be specifically related to previous leprosy reactions. It may be that the time elapsed since reactions obscured their effect. It is also possible that reactions had a more severe effect on peripheral branches.

Studies on the histopathology of nerves in leprosy usually concentrate on the inflammatory component (for review, see Ridley[15]), while a few deal with the neural tissue itself. In early leprosy, Shetty *et al.*[16] described axonal atrophy in cutaneous branches which was attributed to permanent damage to the distal ends of these fibres. However, in active, untreated cases, degeneration with little or no regeneration was observed in the nerve trunks[1,2,3,5,6] as well as in cutaneous branches.[14] The active inflammation and the limited nerve regeneration recorded in these studies are in agreement with our findings. Furthermore, significant nerve regeneration was observed in a single case report, in which previous treatment could not be excluded as a cause.[17] Finally, Dastur *et al.*[4,18] described the presence of inflammatory infiltrate and acid-fast bacilli together with some nerve degeneration and regeneration. However, no information on therapy was included in these reports. The cases reported by Dastur *et al.*[4,18] showed the most severe nerve damage at the predilection sites and peripheral to this segment.

In addition to the striking regeneration, our cases also revealed that the overall histological pattern of the nerve trunks in treated LL and BT leprosy was conspicuously different. In LL cases this homogeneous pattern could be explained by the haematogenous spread of *Mycobacterium leprae*.[19] We observed that occasional small epithelioid granulomata may result from therapy (case 3); however, this did not affect the overall histological pattern. Histological upgrading did not occur in the LL group. In contrast, in

BT cases the uneven distribution of the pathology was in agreement with the views on the fascicular spread of leprosy in this group.[20] In BL cases, the histological pattern was intermediate as represented by cases 6 and 7. However, cases 8–10 showed an increasingly more heterogeneous pattern, which could be explained as upgrading phenomena resulting from therapy.

In summary, the histology reported here was characteristic and of diagnostic value. The most important findings of the present study were the presence of large-scale, but functionally ineffective, regeneration in the ankle region of the posterior tibial nerve of treated leprosy patients, and the lack of complete fibrosis, at least at this level. The cause of this ineffective nerve regeneration is currently being studied in a detailed examination of more peripheral nerve segments, including the innervation of the skin.

Acknowledgements

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Note added in proofs

The osmium-haematoxylin method for paraffin sections and the modified Schoefield's technique, mentioned in the Material and methods, have been published. Miko TL, Gschmeissner SE: Histological methods for assessing myelin sheaths and axons in human nerve trunks. *Biotechnic Histochem* (in press).

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Régénération aux sites lésionnels de prédilection des troncs nerveux dans la lepre traitée

T. L. Miko, S. E. Gschmeissner, C. Le Maitre, Y. Kinfu, R. Kazen et J. H. Pereira

Résumé Il a déjà été démontré que des nerfs périphériques mixtes des membres, de tailles moyene et grande situés en surface, présentent des enflures fusiformes bien reconnues. Il est généralement convenu que ce sont les sites de prédilection des complications nerveuses, c'est-à-dire les sites de dégénérescence et de fibrose les plus graves. Nous avons fait une étude histopathologique semi-quantitative de l'un de ces sites, la région du ligament du fléchisseur du nerf tibial postérieur, chez 14 sujets lépreux traités qui présentaient une perte sensorielle totale du pied depuis 2 à 40 ans. Nous avons fait les observations suivantes: (1) présence d'une régénération nerveuse à grande échelle qui se caractérisait par de nombreuses cellules de Schwann et des axones amyéliniques qui formaient des noyaux de régéneration; (2) les axones myélinques épais étaient soit absents soit en très faibles nombres; (3) la fibrose intraneurale n'était généralement pas grave; (4) la preśence d'inflammation active gênait probablement la régénération nerveuse; (5) cette régénération semble avoir commencé peu de temps après le début du traitement et s'est poursuivie pendant des dizaines d'années; (6) les cas lépromateux se caractérisaient par une pathologie uniformément distribuée tandis que dans les cas tuberculoïdes indéterminés, la pathologie était irrégulièrement distribuée; (7) la régénération nerveuse massive était san efficacité fonctionnelle. Ces résultats indiquent que les lésions nerveuses totales affectent peut-être plus les ramifications nerveuses les plus périphériques.

Regeneración de las áreas predilectas de daño en los troncos nerviosos en la lepra tratada

T. L. Miko, S. E. Gschmeissner, C. Le Maitre, Y Kinfu, R. Kanzen y J. H. Pereira

Resumen Ya se observó que los nervios periféricos mixtos de tamaño grande y mediano y ubicación superficial en las extremidades cuentan con tumefacciones fusiformes. En general se acepta que éstas son áreas predilectas de participación nerviosa en las que so produce la degeneración y fibrosis más severa. Se llevó a cabo un estudio histopatológico semicuantitativo de una de estas áreas, la región del rentináculo del flexor del nervio tibial posterior, en 14 pacientes de lepra tratados, con total pérdida de sensación en el pie durante un período de 4 a 40 años. Se realizaron las siguientes observaciones: (1) presencia de regeneración nerviosa a gran escala, caracterizada por las numerosas células de Schwann y axones no mielinizados que formaban los grupos de regeneración, (2) axones gruesos mielinizados ausentes o presentes en bajo número, (3) la fibrosis intraneural generalmente no es severa, (4) la presencia de inflamación activa probablemente interfirió con la regeneración nerviosa, (5) parece que tal regeneración se inició poco tiempo después de comenzada la terapia y perduró durante décadas, (6) los casos lepromatosos se caracterizaron por una patología de distribución no uniforme, (7) la masiva regeneración nerviosa observada era ineficaz desde el punto de vista funcional. Estos hallazgos indican que el daño nervioso total puede afectar las ramas nerviosas más periféricas.

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The Karonga Prevention Trial: a leprosy and tuberculosis vaccine trial in Northern Malawi. I. Methods of the vaccination phase

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Summary In this report the methods of the Karonga Prevention Trial, a double-blind leprosy and tuberculosis vaccine trial in Karonga District, Northern Malawi, are described in detail. During a total population house-to-house survey, which lasted from November 1985 until August 1989, 121,008 people (57,892 males and 63,116 females) were vaccinated. A further 5835 people refused vaccination and 5757 were ineligible for vaccination, 2652 of them because they had a history or signs of leprosy, or because they were suspected to have early leprosy. A total of 66,145 individuals, without evidence of prior BCG vaccination, received one of the following: BCG, BCG + 5×10^7 killed *Mycobacterium leprae*, or BCG + 6×10^8 killed *M. leprae*; 54,863 individuals found with a typical or a doubtful BCG scar received either placebo or BCG, or (from mid-1987 onwards) BCG + 6×10^8 killed *M. leprae*. Side-effects were not looked for systematically, but 4 individuals self-reported with glandular abscesses, 9 with large post-vaccination ulcers (> 25 mm in diameter) and 2 with ulcers which persisted for more than 1 year.

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BCG vials collected from paraffin refrigerators in the field showed satisfactory concentrations of viable BCG throughout the trial.

Post-vaccination skin test (RT23 and *M. leprae* soluble antigen) results and post-vaccination ulcer rates indicate that few mistakes were made in the field when recording the vaccine codes.

Introduction

There are currently 4 major trials under way to investigate the protective efficacy of different potential vaccines against leprosy.[1,2,3,4] Each of these trials has a unique design. All include BCG in at least 1 comparison group, but dosages and manufacturers differ and 3 trials, in Venezuela, Malawi and South India, include combined BCG plus killed *M. leprae* vaccines.

Taking into account previous experience with BCG trials against leprosy (and tuberculosis) it is expected that the results of the current trials will differ in various ways.[5] In order to be able to interpret such differences as may arise, it is necessary to consider in detail the methods used in each trial. In this report we describe the procedures employed in the vaccination phase (1986–89) of the Karonga Prevention Trial (KPT), a leprosy and tuberculosis vaccine trial in Karonga District, Northern Malawi.

The Karonga Prevention Trial was designed to test the following hypotheses:

(i) adding killed *M. leprae* to BCG increases the protective efficacy of BCG vaccine against clinical leprosy; and
(ii) repeated BCG vaccination increases the protective efficacy of BCG vaccine against both clinical leprosy and tuberculosis.

Methods

HISTORICAL BACKGROUND

A longitudinal study of the epidemiology of leprosy, known as the LEPRA Evaluation Project (LEP), was initiated in Karonga District in 1979.[6] The design of that study required 2 total population surveys in order to relate incident leprosy and tuberculosis cases discovered at the 2nd survey to information on suspected risk factors collected at the 1st survey. After a pilot study in 1979, the first house-to-house survey was carried out from April 1980 to August 1984. It was then decided to combine the 2nd survey of the LEP with the intake phase of a leprosy and tuberculosis vaccine trial.[2]

Trial area

Karonga District covers an area of 3346 sq. km along the north end of Lake Malawi (Figure 1). According to the preliminary report of the 1987 population and housing census the total population of Karonga District was 147,096 in September 1987.[7] A small, hilly, sparsely populated section of Karonga District projecting into Chitipa District was omitted from the intake phase of the trial just as it had been from the 1st total population survey of the LEP.[6] Sensitization and dosage studies were carried out in 1984[8] and at the

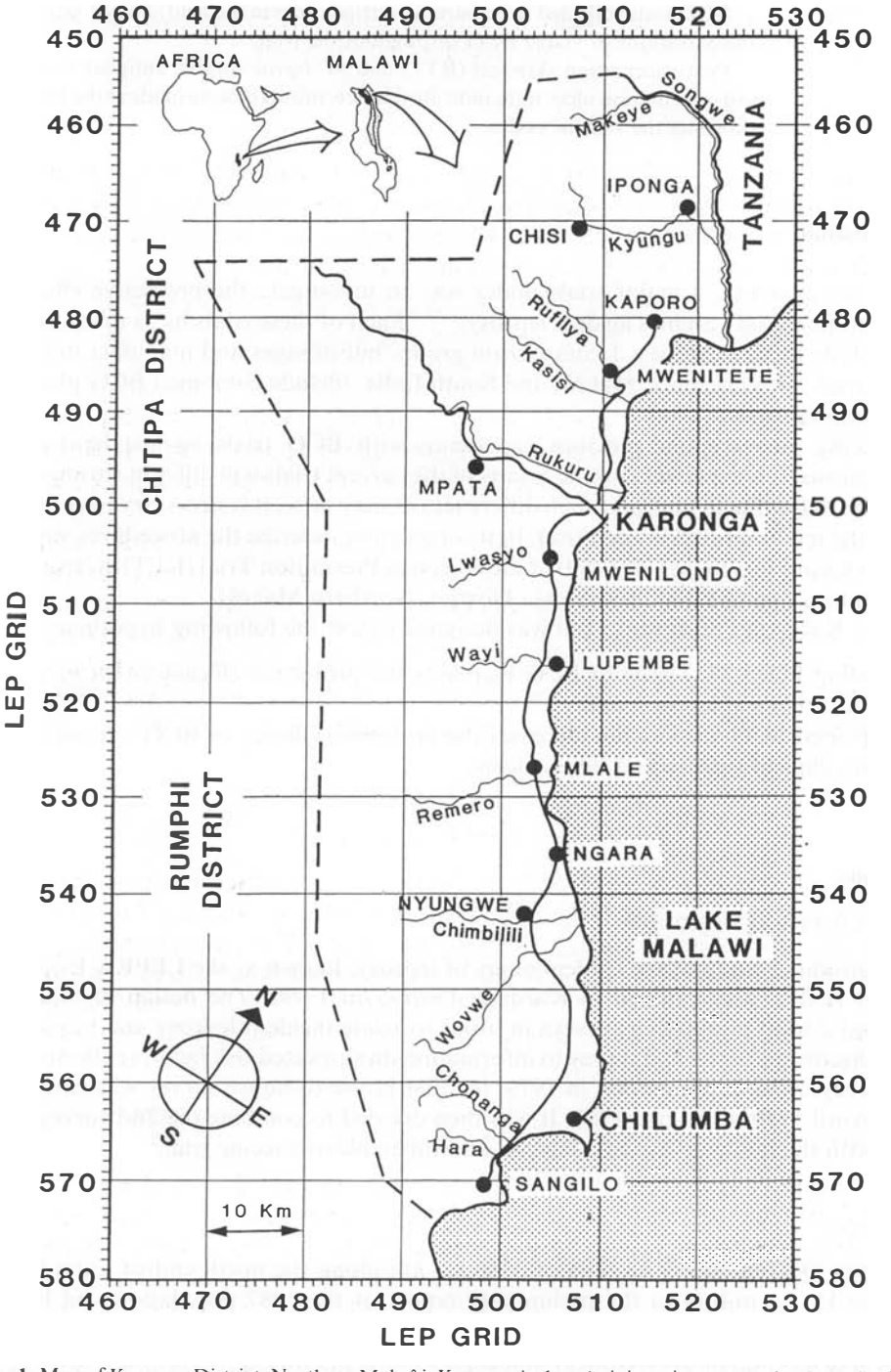

Figure 1. Map of Karonga District, Northern Malawi. Karonga is the administrative centre (the 'Boma') of the district. Headquarters of the Karonga Prevention Trial are in Chilumba.

end of 1985 in several villages in the vicinity of Chilumba, the project headquarters, and so these villages were also not available for the main trial intake.

Trial design

The general design of the trial has been described elsewhere.[2] People eligible for vaccination and who agreed to participate were divided according to their BCG scar status, which was determined at an examination by Leprosy Control Assistants (LCAs).

Individuals without a BCG scar were allocated to either

BCG or
BCG + 5×10^7 killed *M. leprae* or
BCG + 6×10^8 killed *M. leprae*.

Thus all BCG scar negative individuals were given at least BCG. The BCG component of the scar negative vaccine was therefore labelled as such and only the dilutent (either water, or a suspension of 5×10^8, or 6×10^9 killed *M. leprae*/ml), which was supplied in brown vials, was code-labelled.

Individuals with a BCG scar, and those who BCG scar was considered doubtful by the LCAs (both called 'scar positives' hereafter), were, until mid-1987, given either BCG or a placebo. Therefore, for this group the dilutent was always water and the blind component of the vaccine was either BCG or dextran, provided as identical-looking freeze-dried pellets in identical-looking code-labelled vials. From mid-1987 onwards BCG + 6×10^9 killed *M. leprae*/ml was added as a 3rd arm for BCG scar positive individuals. Scar positive individuals were thus allocated to receive either

placebo or
BCG or
BCG + 6×10^8 killed *M. leprae*.

This made it necessary to match pellet and diluent so that no individual received killed *M. leprae* only (dextran pellet plus killed *M. leprae* diluent). Vaccines for scar-positive individuals were therefore supplied paired in small self-sealing plastic bags. This, incidentally, meant that BCG for scar positives was stored frozen (because it was paired with killed *M. leprae* bottles) from mid-1987 onwards, while BCG (labelled as such) for scar negative individuals continued to be stored at +4°C in solar refrigerators in Chilumba.

Vaccines

All BCG used in the trial was supplied free of charge by Glaxo. Lot numbers of BCG and dates of dispatch to Malawi are listed in Table 1. Further BCG lots were supplied by Evans from September 1989 onwards for the vaccination of babies born after the intake phase of the KPT had been completed (see below). BCG vials were collected from field refrigerators and taken to the UK for quality control whenever this was feasible, e.g. by the IMMLEP appointed trial monitors (initially MCP, later DC) during their visits. Quality control was carried out by Dr P. A. Jenkins at the Mycobacterium Reference Unit of the Public Health Laboratory Service, University Hospital of Wales, Cardiff.

M. leprae were derived from armadillos inoculated either with *M. leprae* direct from

Table 1. BCG lot numbers used in the trial and their times of dispatch to Malawi

Shipment	Month and year of dispatch	Lot number (manufacturer's number)	Number of (10-dose) vials
1	December, 1985	D752	720
2	March, 1986	D763	720
		D747	65
3	July, 1986	D763	1100
4	November, 1986	D763C	1200
5	March, 1987	D763C	1000
6	August, 1987	D907	900
7	October, 1987	D907	900
8	February, 1988	D907	900
9	May, 1988	D907	320
10	July, 1988	D907	1000
11	September, 1988	D1184	800
12	January, 1989	D1184	1300
13	March, 1989	D1188	800
14	June, 1989	D1188	1000
15	August, 1989	D1188	1000
Total			13725

patients or with *M. leprae* passaged not more than twice in other armadillos. The vaccine was prepared in licensed premises at Wellcome Laboratories (Beckenham, Kent, UK), under contract to IMMLEP and under supervision of the IMMLEP Bank. Only *M. leprae*-infected liver was used, this being the richest and most easily processed tissue. The livers chosen were sterile, free from cultivable mycobacteria and were exposed to 2·5 mega rad gamma radiation from a ^{60}Co source to ensure that all *M. leprae* were killed. Extraction and purification of *M. leprae* followed IMMLEP Protocol 1/79.[9] Each 50 g liver preparation yielded approximately 10 ml suspension of purified *M. leprae*, from which 0·5 ml was used for a quantitative and qualitative assessment of its suitability for vaccine use. From time to time preparations were pooled into lots as required to sustain the trial. After the volume and density of the lot was determined it was diluted with 0·01% Tween 80/phosphate-buffered saline to the concentration required for the trial (i.e. 5×10^8 and 6×10^9 AFB/ml). These suspensions were aliquoted in 1·1 ml quantities into 4 ml rubber capped brown bottles, sealed, autoclaved at 120°C for 20 minutes and stored at −70°C.

In all, 3 lots of vaccine (II–IV) were required for the trial in Malawi. Lot II was also used in the leprosy vaccine trial in Venezuela and the remainder of Lot IV will be used in the trial in South India.

All vaccines except BCG vials labelled as such were sent to Malawi on dry ice. BCG vials were usually shipped on wet ice.

Randomization

All vaccine vials were coded by the IMMLEP-appointed trial monitors (MCP, DC). The code included a check letter to enable the identification of key punch errors at the time of data entry. In addition, the range of code numbers was different for scar-positive and scar-

negative vaccines. This helped quality control in Chilumba because data entry programs did not permit routine entry of records with a discrepancy between BCG scar status and type of vaccine given. Such records could only be entered by making use of a password.

As multi-dose vials were used, most vaccine codes apply to several (up to 11) individual recipients from the same vial.

Field organization

Initially 5 teams (later increased to 6 and, towards the end of the intake phase, 7) operated in the field. Each field team was composed of 1 interviewer, 2 or 3 LCAs and 2 vaccinators. A cook was employed for each team at their camp. In addition a villager, usually a relative of the village headman or someone recommended by him, was employed as a 'motivator' to remind people the previous evening that the team would visit them the following day according to prior arrangements.

Interviewers carried A5 size computer print-outs ('field tickets') for all individuals found during the 1st total population survey in a particular village. After identifying an individual the interviewers either completed the field ticket for that person (and made corrections to the print-out if necessary) or filled a new personal form if no field ticket was available because, for example, the person was new in this village. Anyone vaccinated previously in the trial was meant to be excluded from (another) vaccination by the interviewers (see Figure 2). Interviewers also looked for tuberculosis cases, and sputum specimens were collected from anyone who reported a productive cough of more than 3 weeks' duration. Specimens were stored at 4–10°C in the camp refrigerators before being sent to the project laboratory in Chilumba every week.

Until the end of 1985 sputum specimens were examined only by microscopy in Chilumba. *M. tuberculosis* culture facilities were introduced in Chilumba early in 1986, in collaboration with the Mycobacterium Reference Unit of the Public Health Laboratory Service, in Cardiff. Details will be described separately.

Usually people were first interviewed and then, during a thorough examination for any signs of leprosy, LCAs decided whether or not an individual was still eligible for vaccination (Figure 2). LCAs were meant to exclude known leprosy patients, leprosy suspects, malnourished individuals, in particular malnourished children, and anyone else seriously ill. Individuals born before 1914 and visitors from outside Karonga District were only vaccinated at their request. They were not encouraged to come forward for vaccination. Similarly, babies who were under 3 months old were only vaccinated at the request of their parents, otherwise they were not included in the trial. Children with an acute febrile illness, for example malaria or measles, were vaccinated after they had recovered.

Each team had a paraffin refrigerator and 2 spare paraffin refrigerators were kept at headquarters. BCG vaccines for scar-negative individuals, and, until mid-1987, the pellets and diluent for scar-positive individuals, were stored in the cold compartment (4°C–10°C depending on the time of the year). Dilutent for scar-negative individuals and vaccines for scar-positives from mid-1987 onwards were stored in the freezer compartment. Vaccines were supplied to the teams from Chilumba on a weekly basis. They were delivered in vaccine carriers ('Thermos' vaccine carrier class B, model 3504/38). Once vials had thawed, they were not put back into the freezer compartments.

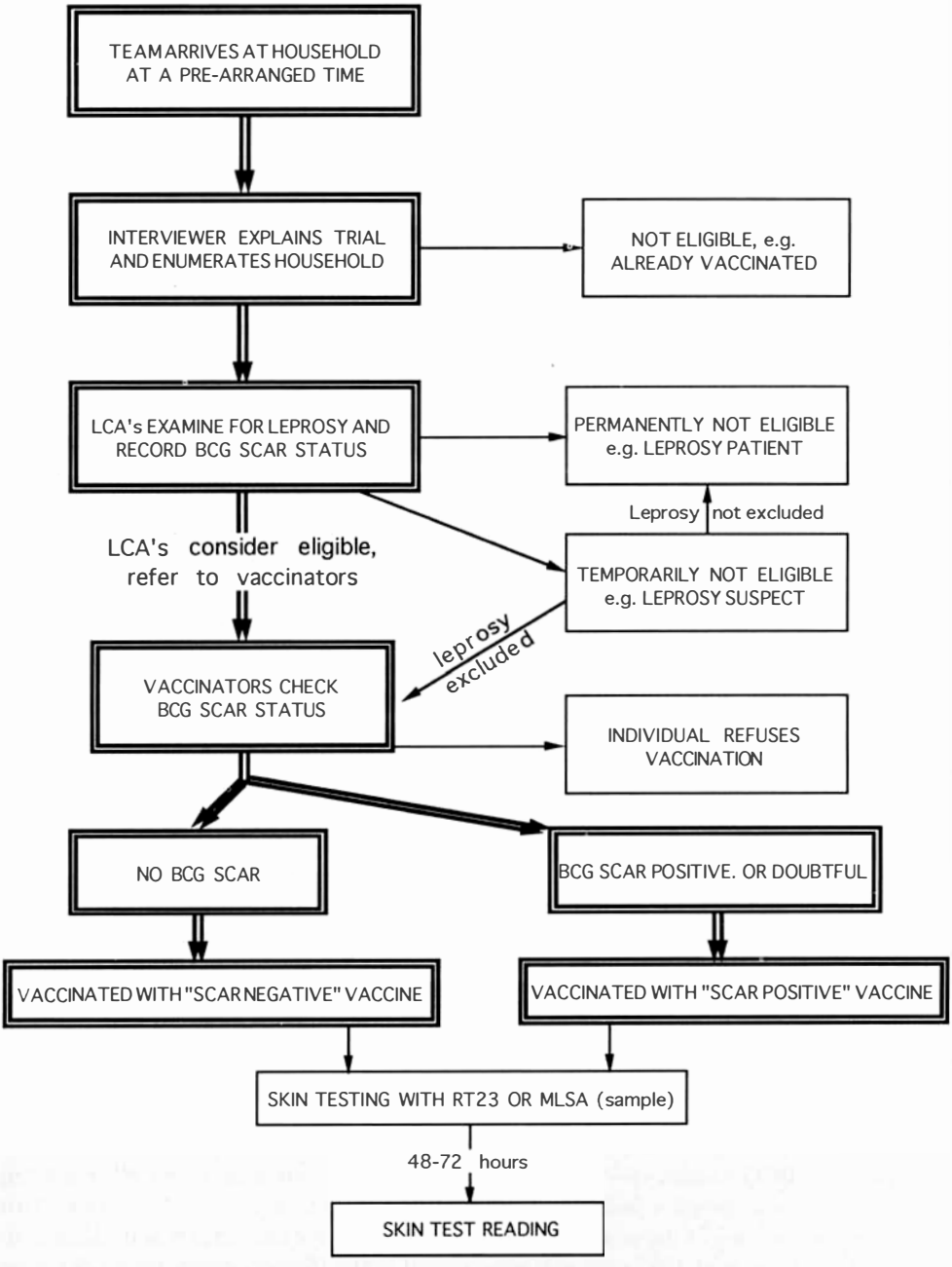

Figure 2. Field activities during the recruitment phase of the Karonga Prevention Trial, 1986–89.

At the beginning of the trial each vaccine code was copied by the vaccinators from the vial used onto the vaccinated individual's examination form. However, it was soon realized that vaccinators sometimes either forgot to write down the codes or made errors when copying. Double entry of codes into registers did not entirely solve the problem. The vaccines were therefore packed with 10 preprinted labels which the vaccinators only had to stick onto the examination forms after vaccination.

The vaccinators carried the vaccines to the field in wide-mouthed Thermos flasks containing ice cubes. They reconstituted the vaccines after arriving at the first household scheduled for examination and vaccination each day. For BCG scar negatives the BCG vial was put into a black sleeve, opened, and the BCG was suspended in 1 ml diluent which had been drawn with a tuberculin syringe from the next brown vial in the Thermos flask. Initially the same tuberculin syringe was also used for injection, being sterilized by flaming after each injection of 0·1 ml vaccine. As some vaccine had to be squirted out after flaming the needle, only 8 or 9 doses were obtained from each 1 ml vial of vaccine. Both the syringe and the needle were discarded after the contents of the syringe had been finished. During the latter half of 1987, sterilizable 0·1 ml syringes and needles were introduced, following a recommendation by the trial monitor and the Leprosy Unit of the WHO. This meant that the reconstituted BCG had to be re-injected into the brown diluent vial and drawn up 1 dose at a time. Because all of the 1·1 ml diluent was now used to reconstitute the 1 mg BCG this meant that vaccinees received on average only 0·09 mg BCG rather than 0·1 mg as previously. A separate syringe and needle were used for each vaccinee. Needles and syringes were steam-sterilized at the end of each day at the camp following standard EPI procedures.[10]

For BCG scar positives the process was similar until mid-1987, but from then on vaccinators had to be careful to use pellet and diluent as matched. They were instructed to compare labels on the powder and diluent carefully and to be sure that the code numbers were identical and the same as those on the spare labels provided for the forms.

Vaccinators were not allowed to reconstitute the next vial of scar-negative or scar-positive vaccine until the last vial had been completely finished. There were strict instructions to tear up any unused labels after each vial was finished. Reconstituted vaccines not used by the end of the day were discarded. To further minimize confusion, vaccinators not only used differently coloured flasks for scar-negative and scar-positive vaccines but were also given red nail varnish to mark vials and syringes for scar-positive vaccines. The colour coding extended to labels—those for scar-positive vaccines were pink, and those for scar-negative vaccines were white.

Vaccinators were trained for 2–3 months before they were allowed to vaccinate. During the training period they injected 0·1 ml skin test reagents, RT23 tuberculin or *M. leprae* soluble antigen preparations, intracutaneously in the volar surface of the forearm. The tuberculin (RT23, 2IU per 0·1 ml dose) was purchased from the Statens Serum Institute, Copenhagen, Denmark. *M. leprae* soluble antigen skin test reagents were prepared at the National Institute of Medical Research, London.[11] Skin test induration sizes were read on the 2nd or 3rd day after injection. Induration diameters were measured along and across the arm and both numbers were recorded, although only the mean diameter was coded. In some areas vaccinators collected fingerprick blood specimens, dried on chromatography paper, instead of carrying out skin tests. A full analysis of skin test and serology results, before and after vaccination, will be published separately.

All vaccines were injected intradermally into the right deltoid region, the area used for

BCG vaccination in Malawi. A different site was chosen only in exceptional circumstances e.g. the volar side of the left forearm.

The vaccinators tried to persuade all eligible individuals to agree to vaccination. People were concerned mostly about ulcers, particularly whether they might hinder them when it was time to plant, weed or harvest. It was explained to people that the purpose of the trial was to find a vaccine against leprosy, and that everyone who developed an ulcer would benefit to some degree from the vaccination, but that some of the vaccines we were using might be stronger than others. Initially public meetings were held to explain our work, but village headmen soon said that people knew all about us and that there was no need for further meetings. Village headmen and elders were always briefed before a team moved into their area.

People were not reviewed routinely for side effects after vaccination but were encouraged to self-report to a team if they had any untoward reaction.

Several times during the intake phase the vaccinators were required to measure vaccine ulcers in individuals vaccinated 4 weeks previously. This involved removal of the scab with an alcohol swab and measurement of the inner diameters of the ulcer along and across the arm. The mean diameter was coded and used for analysis. All ulcers on the right deltoid area were assumed to be attributable to the vaccination, even if they did not look like typical vaccine ulcers. No history was taken by the vaccinators when measuring ulcers. Ulcer sizes recorded by one particular vaccinator were excluded from the analysis after it was discovered that some of his results were invented and not based on actual measurements.

Health centre based activities

Towards the end of the trial intake phase vaccinators were also posted to various rural health centres and to the district hospital in Karonga. They vaccinated babies attending the under-5s clinics who had been born or who had arrived with their families after a field team had been in their village. In addition, these vaccinators also collected sputum specimens from anyone self-reporting with a productive cough of more than 3 weeks' duration (as done by the interviewers in the field).

Vaccinators at the health centres were supplied with up-to-date computer print-outs of all children already vaccinated in the trial, in order to avoid accidental re-vaccination of children. In cases of doubt the vaccinators contacted project headquarters to find out whether or not a particular child had already been vaccinated. Up to June 1990 the vaccinators used Glaxo BCG alone for babies under 3-months-old, but randomized 'scar negative' vaccines for those over 3-months-old. From June 1990 onwards the vaccinators used Glaxo/Evans BCG for every child. Irrespective of age the infants received 0·1 ml vaccine in the right deltoid area.

Data processing

All general examination forms and household and personal questionnaires were coded, checked and entered on Burroughs B26 microcomputers in Chilumba. Entry programs, written in COBOL, contained a large number of range and consistency checks and some variables were entered twice on different screens. However, the systematic double entry of *all* questionnaires and examination forms was not attempted because it was thought that

Table 2. Numbers of individuals vaccinated once during the intake phase of the leprosy vaccine trial in Karonga District by age, sex and type of vaccine. One individual vaccinated but with an unknown year of birth has been excluded from this table

Age at vaccination	Scar-negative vaccine		Scar-positive vaccine		Total
	Males	Females	Males	Females	
<5	6562	6619	3525	3312	20018
5–14	7716	8006	11949	11232	38903
15–24	3739	4193	6703	7277	21912
25–34	2304	4369	3257	3622	13552
35–54	6548	8602	1189	1769	18108
≥55	3862	3625	538	490	8515
Total	30731	35414	27161	27702	121008

this would make the data handling procedures too cumbersome. Approximately every month all complete data were copied onto floppy disks: 1 set of disks was sent to London, and 1 was retained in Chilumba while deleting the data from the hard disks. In comparison with the procedures used during the 1st total population survey,[6] the entry of data in Chilumba had many advantages. In particular, entry and check programs could easily be updated when new variables or codes were introduced. Local data entry also facilitated verification of information rejected by the check programs at the time of data entry. As during the first total population survey, details of leprosy and tuberculosis patients and serology results were coded on special forms and sent to London for entry.

In London all data were transferred to the Amdahl mainframe of the University of London Computer Centre.

Analysis

We here present data on the numbers of people who were vaccinated, the numbers who were eligible but refused vaccination and the numbers not eligible. For individuals seen more than once the first recorded reason for refusal or non-eligibility is used, except in the case of leprosy suspects for whom the reason for non-eligibility is taken from the 'review examination' form filled in by a medical officer. All analyses requiring the breaking of the vaccine codes were and are carried out by the trial monitors (MCP, DC).

All babies vaccinated at the under-5s clinics are excluded from the following analyses irrespective of the type of vaccine they received. In so far as they were *brought* to the health centres they are different from the babies recruited at their homes, and thus it is less appropriate to calculate refusal rates for them. Also excluded from the analyses are 298 individuals who were accidentally vaccinated twice and 2 who were vaccinated 3 times.

Results

TRIAL POPULATION

Table 2 shows the numbers of individuals who were vaccinated once during the main intake phase by age, sex and type of vaccine they received. The total number of people

Table 3. Numbers of individuals ineligible for vaccination during the intake phase of the leprosy vaccine trial in Karonga District by reason and by age

	Reason for non-eligibility at first examination						
Age	Age < 3 months	Malnutrition	Seriously sick	Born < 1914	Leprosy	'Already vaccinated*'	Total
< 5	657	425	167	—	0	3	1252
5–14	—	51	93	—	141	7	292
15–24	—	1	54	—	407	13	475
25–34	—	0	47	—	445	7	499
35–54	—	0	78	—	985	5	1068
> 54	—	2	149	1339	674	7	2171
Total	657 (11·4)†	479 (8·3)	588 (10·2)	1339 (23·3)	2652 (46·1)	42 (0·7)	5757 (100)

* Wrongly assumed to have already been vaccinated by another team in another village.
† Numbers in parentheses are percentages.

recruited into the trial was 121,008, of whom 66,145 received 'scar-negative' and 54,863 'scar positive' vaccines. One individual who was vaccinated but whose year of birth was not recorded has been excluded from this table.

Table 3 shows the distribution by age of the 6 different reasons why 5757 individuals were considered ineligible for vaccination. One individual whose year of birth was not recorded is excluded from this table. Of the 5757 ineligible individuals 3177 (55%) were female and 2580 (45%) male. The interviewers were persuaded by 42 individuals that they had been vaccinated earlier in some other village and by the time it was discovered that they had not it was no longer practical to go back to their households to vaccinate them. We excluded 2652 (46%) ineligible individuals because of a history of leprosy, residual signs of leprosy, active leprosy or a suspicion of leprosy even if not confirmed. Although a history of leprosy was a reason for ineligibility, we identified 280 individuals with a history of leprosy but without residual signs who were accidentally vaccinated because they did not mention to the LCAs that they had once received or were receiving anti-leprosy treatment. In general their histories were only discovered in the office long after they had been vaccinated. A mild type-1 reaction developed in a previously treated patient soon after vaccination.

Tables 4a and 4b show the reasons why 3132 females and 2703 males refused vaccination. Vimbuza is the local term for a condition in which the individual is 'possessed by spirits'. In general these spirits do not allow the person they possess to receive any injection. If someone possessed by spirits is given an injection a violent attack may result, usually taking the form of guttural cries, associated with uncontrollable jerking of the body, and sometimes followed by a period of loss of consciousness. Vimbuza is known to be more common among females than males[12] and this is apparent from Tables 4a and 4b. Vimbuza was cited as the reason for refusal by 35·2% (1104/3132) female refusers but by only 19·2% (519/2703) of male refusers. The term 'vimbuza' also includes 'mitima', 'mizimi', 'visilisi' and 'chikoko', although the terms have somewhat different meanings. 'Visilisi' are particularly violent spirits while the term 'chikoko' when used for children often means a history of febrile convulsions rather than possessions by spirits. Also 90 members of fundamentalist religious communities, in particular the St Michael's Church,

Table 4a. Number of females who refused vaccination during the intake phase of the leprosy vaccine trial in Karonga District by age and reason given (if any)

	Reason for refusal to be vaccinated (if any)					
Age	Vimbuza	Religious	Mentally ill	Others	No reason*	Total
< 5	103 (chikoko)	1	0	12	272	388
5–14	87	8	1	4	176	276
15–24	138	9	6	6	420	579
25–34	166	3	8	3	328	508
35–54	396	6	6	17	423	848
≥55	214	3	3	15	298	533
Total	1104 (35·3)†	30 (1·0)	24 (0·8)	57 (1·8)	1917 (61·2)	3132 (100)

* Individuals who refused to be vaccinated without giving a reason.
† Numbers in parentheses are percentages.

Table 4b. Number of males who refused vaccination during the intake phase of the leprosy vaccine trial in Karonga District by age and reason given (if any)

	Reasons for refusal to be vaccinated (if any)					
Age	Vimbuza	Religious	Mentally ill	Others	No reason*	Total
< 5	110 (chikoko)	11	1	6	245	373
5–14	84	14	5	14	199	316
15–24	78	8	6	8	424	524
25–34	80	8	9	10	493	600
35–54	122	14	8	8	448	600
≥55	45	5	3	8	229	290
Total	519 (19·2)†	60 (2·2)	32 (1·2)	54 (2·0)	2038 (75·4)	2703 (100)

* Individuals who refused to be vaccinated without giving a reason.
† Numbers in parentheses are percentages.

refused vaccination. This may be an underestimate because members of the St Michael Church were generally unco-operative and we may have under-recorded their number. 'Others' stands for a variety of reasons, such as fear of keloid scars. We could have recorded the 56 severely mentally ill individuals as 'ineligible', since they were not able to give consent. Although 3955 individuals refused vaccination, simply stating that they did not want to be vaccinated.

QUALITY CONTROL MEASUREMENTS

Figures 3a and 3b show relative frequency distributions of diameters of 1499 ulcers among scar-negative and scar-positive individuals broken down by vaccine type. The

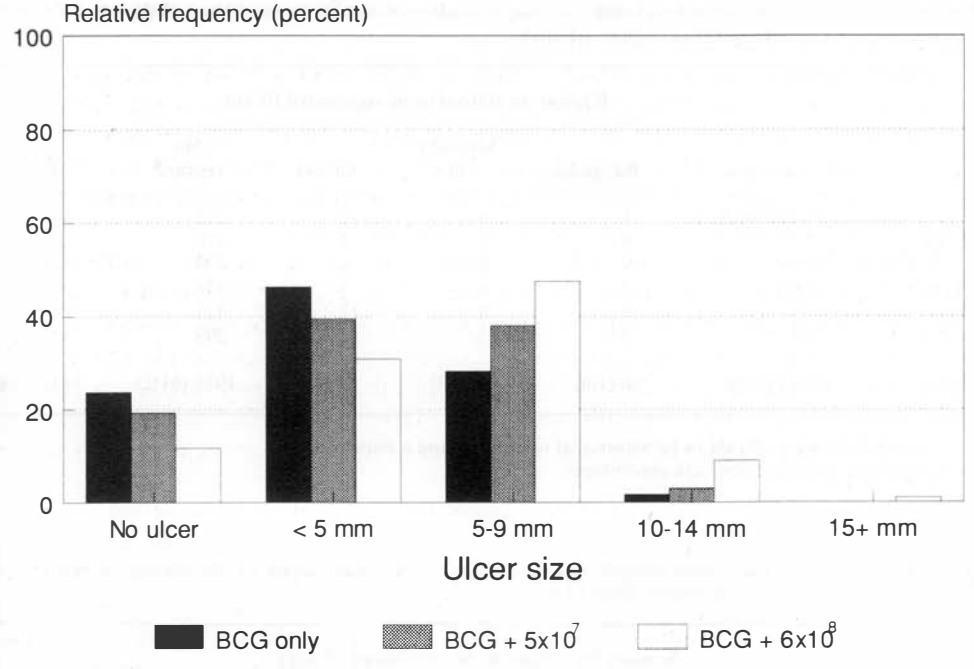

Figure 3(a). Ulcer sizes in BCG scar-negative individuals 4 weeks after vaccination, by type of vaccine given. All 794 vaccinees received at least BCG.

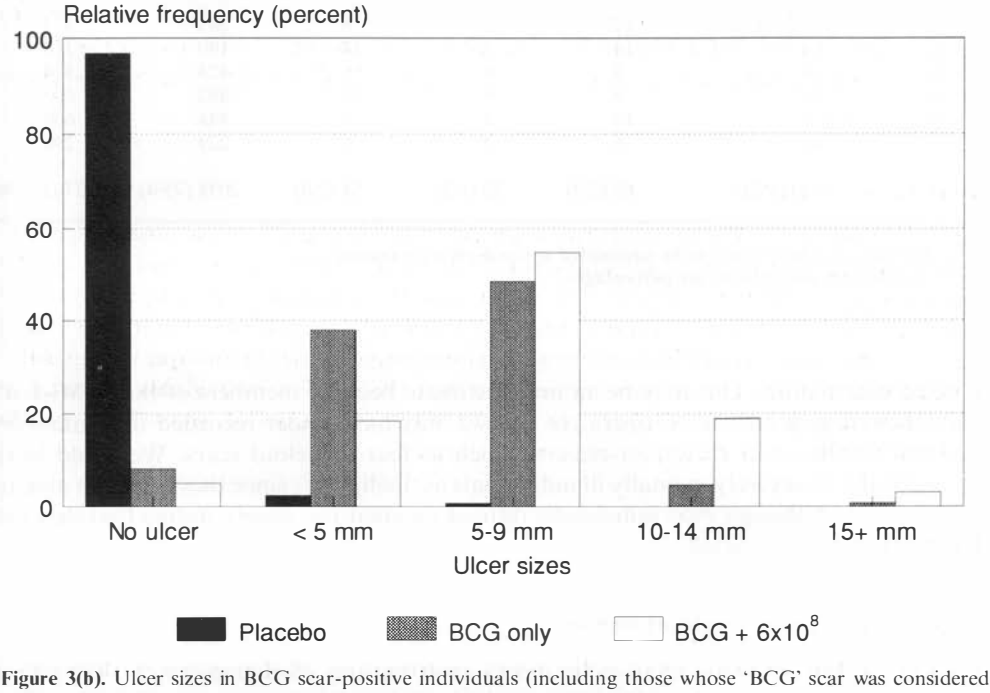

Figure 3(b). Ulcer sizes in BCG scar-positive individuals (including those whose 'BCG' scar was considered doubtful) 4 weeks after vaccination, by type of vaccine given. Of the 705 vaccinees 248 received placebo only.

average ulcer size increased with increasing the dose of killed *M. leprae*. Age and sex had little influence on these ulcer sizes (not shown).

Figures 4a and 4b show cumulative frequencies of ulcer sizes by initial scar status. Among scar negatives, ulcers over 10 mm in diameter were observed in 1·0% (3/291) who were given BCG only, 2·5% (5/202) of those given BCG + 5×10^7 and in 5·0% (15/301) of those given BCG + 6×10^8 killed *M. leprae*. Among BCG scar positives 3·5% (9/256) of those given BCG only were found with an ulcer over 10 mm in diameter compared to 14·9% (30/201) of those given BCG + 6×10^8 killed *M. leprae*.

Table 5 shows skin test results from 433 vaccinees tested with RT23 tuberculin or MLSA (batch CD19) at the time of vaccination and approximately 3 months thereafter. The average increase in tuberculin sensitivity was similar among all groups receiving BCG, with or without killed *M. leprae*. On the other hand, the increase in sensitivity to CD19 was correlated with the concentration of killed *M. leprae* in the vaccine. Of particular relevance (for the purpose of this paper) is the fact that there was no appreciable change in the average skin test induration sizes among vaccinees who received placebo only.

According to the records, 63 BCG scar-negative individuals received 'scar-positive' vaccine. In addition, 39 individuals with a definite BCG scar, and 44 individuals with a doubtful BCG scar, were mistakenly given 'scar-negative' vaccine. In addition the BCG scar status was not recorded for 7 vaccinees. There are 3 possible explanations for the discrepancies between BCG scar status and type of vaccine given: (i) an appropriate vaccine was given but the scar was wrongly recorded; (ii) an appropriate vaccine was given but the wrong label was attached to the individual's General Examination Form; or (iii) an inappropriate vaccine was indeed given.

During the intake phase of the KPT, 57 vials of BCG were taken back to the UK for quality control assessment. Figure 5 shows the distribution of counts of viable BCG per vial. The counts range from $2{\cdot}5 \times 10^6$ to 1×10^8 viable BCG per vial. The median count was $2{\cdot}4 \times 10^7$.

ADVERSE REACTIONS

Apart from large (> 10 mm) ulcers (see Figures 3a, 3b, 4a and 4b), a few other side effects were observed or brought to the attention of the project staff. These included glandular abscesses (4), exceedingly large (> 25 mm) ulcers (9) and ulcers persisting more than 1 year (2). There was 1 hypersensitivity reaction probably attributable to dextran in a BCG scar-positive child.[13] In addition, there was an unexplained series of multiple though small vaccine ulcers which caused some concern in mid-1986. A few individuals informed us that they had gone to Karonga District Hospital because of side-effects of the vaccinations but their records could not be traced.

Discussion

We emphasize here issues related to the allocation of the vaccines in the field and to quality control of the vaccines.

There is evidence that BCG vials collected from the paraffin refrigerators in the field contained satisfactory numbers of viable BCG. All 57 vials which were sent for quality

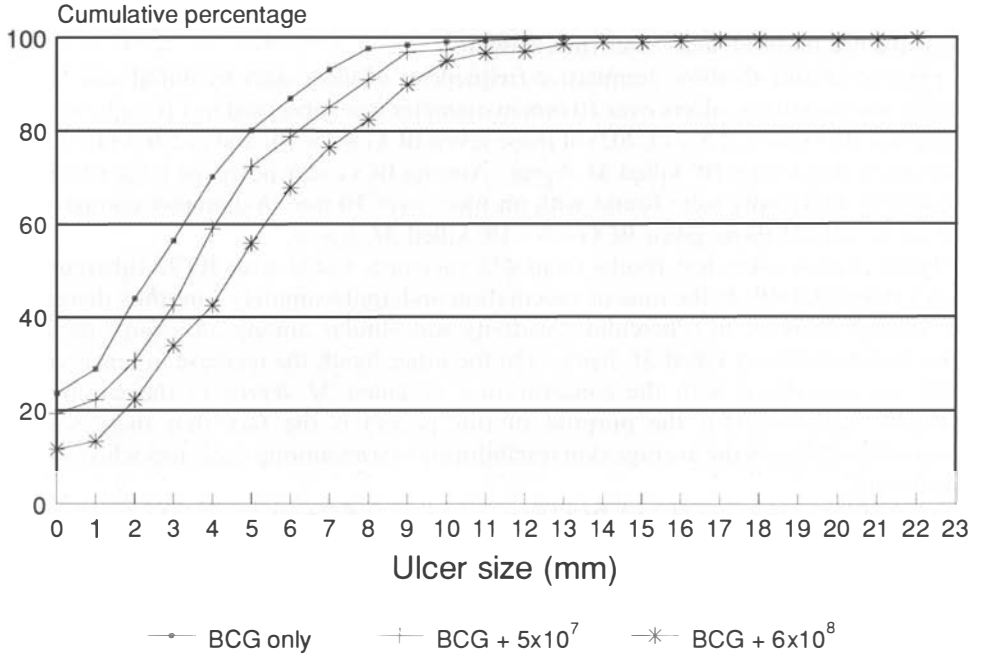

Figure 4(a). Cumulative frequency of ulcer sizes in BCG scar-negative individuals 4 weeks after vaccination, by type of vaccine given. All 794 vaccinees received at least BCG.

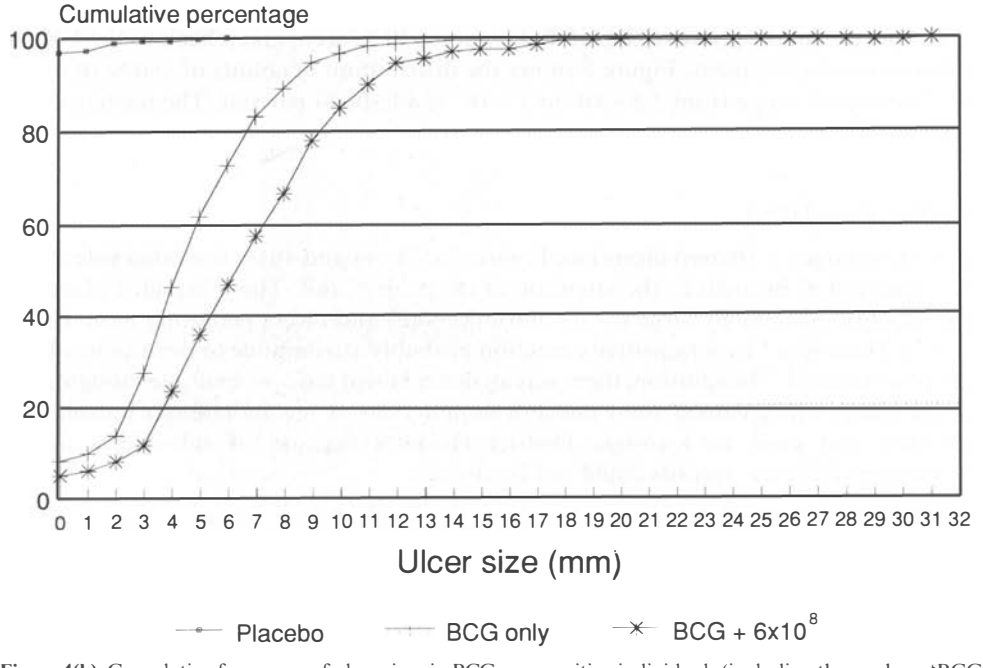

Figure 4(b). Cumulative frequency of ulcer sizes in BCG scar positive individuals (including those whose 'BCG' scar was considered doubtful) 4 weeks after vaccination, by type of vaccine given. Of the 705 vaccinees 248 received placebo only.

Table 5. Pre- and post-vaccination skin test results (average induration diameter) in 433 vaccinees tested in 1987 (before the introduction of the 3rd arm among BCG scar positives). RT23 = batch number of Statens Serum Institute tuberculin, CD19 = batch number of *M. leprae* soluble antigen skin test reagent used. KMl = Killed *M. leprae*

Antigen	Vaccine	Number tested	BCG scar status of those tested	Pre-vaccination x_1 (SD)	Post-vaccination x_2 (SD)	Change x_2-x_1 (SE)
RT23	BCG	83	negative	7·0 (7·4)	12·0 (6·2)	+5·0 (5·8)
	BCG + 5×10^7 KMI	25	negative	5·0 (6·5)	10·8 (5·8)	+5·8 (5·6)
	BCG + 6×10^8 KMI	69	negative	6·8 (7·1)	11·0 (5·4)	+4·2 (6·4)
	BCG	64	positive	5·9 (5·6)	11·9 (5·3)	+6·0 (4·7)
	Placebo	72	positive	5·3 (5·8)	5·9 (5·9)	+0·6 (4·2)
CD19	BCG	41	negative	3·0 (5·3)	6·2 (6·2)	+3·2 (8·4)
	BCG + 5×10^7 KMI	5	negative	2·0 (4·5)	6·6 (7·8)	+4·6 (5·8)
	BCG + 6×10^8 KMI	15	negative	1·7 (4·4)	7·7 (8·6)	+6·0 (10·1)
	BCG	25	positive	3·1 (6·0)	5·7 (7·2)	+2·6 (9·5)
	Placebo	34	positive	4·4 (6·0)	3·9 (5·9)	−0·6 (7·4)
Total		433				

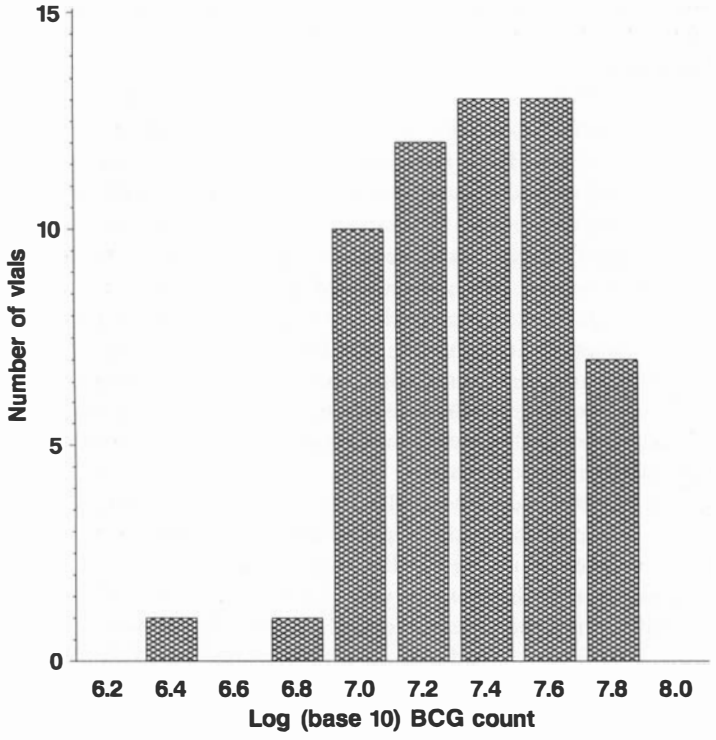

Figure 5. Counts of viable BCG in 57 vials collected from paraffin fridges in the field during the intake phase of the Karonga Prevention Trial (1986–89). Counts are per vial, thus per 10 doses.

control were collected from the field and the field staff were not aware of the timing and the purpose of the collection. There is no reason to believe that the staff responsible for the cold chain (office staff, drivers and vaccinators) took more care than usual concerning the delivery and storage of vaccines during the weeks preceding the collection of BCG vials.

Of real concern is how often a wrong label was copied or stuck onto an examination form. All unused code labels were supposed to be destroyed after each vial was finished, and before reconstitution of the next vial. As only 2 vials were open at any time, mistakes should only have occurred when the vaccinator stuck a scar-positive label onto an examination form even though a scar-negative vaccine had been given, and vice-versa. The data on post-vaccination skin-test induration sizes in the placebo group (Table 5) show no evidence for such errors. On the other hand, of 248 BCG scar-positive individuals who were recorded as having received a placebo injection, and who were examined for an ulcer 4 weeks after vaccination, 8 were recorded as having an ulcer in the right deltoid area.

Are there explanations apart from the wrong labels being used in these 8 instances? None of the other individuals who had been vaccinated from the same (8) vials was observed to have developed an ulcer; thus there is no indication that the vials themselves were mislabelled. This leaves 3 alternative explanations: (i) the ulcers, in particular the 6 ulcers which measured 1–3 mm in diameter, were not vaccine ulcers but represent injuries or abrasions, perhaps after insect bites; (ii) individuals were wrongly identified at the time of measuring the ulcers; or (iii) staff invented the ulcer sizes instead of going, maybe a long way, to find the individuals they had been asked to examine for vaccine ulcers. The last explanation cannot be discounted even though the 8 ulcers were all recorded by different staff. Given personal experience (JMP) of mistaking an ordinary ulcer (abrasion) for a vaccine ulcer, the first explanation would seem the likeliest explanation for most of the ulcers found 4 weeks after vaccination in individuals allocated to placebo only.

A discrepancy between BCG scar status and vaccine given was found in 153 ($63 + 44 + 39 + 7$, see above) individuals. Such errors may have arisen in several ways. For example, if a vaccinator went alone to vaccinate a child after he/she had recovered from measles, the vaccinator might have seen no obvious BCG vaccination scar and overlooked the fact that the LCA had recorded that there was a (doubtful) scar. If the label of the vaccine actually used was stuck onto the examination form, such mistakes do not matter greatly. However, it is possible that for some of these 153 individuals a wrong vaccine code label was attached to the form. Any such mistakes will lead in theory to a reduction of observed protective efficacies of the various vaccines employed in the trial.

Some individuals were undoubtedly misclassified concerning their BCG scar status,[14] but their number will never be known.

Approximately 121,000 people were ultimately recruited into the Karonga Prevention Trial, which is very close to the number predicted.[2] The numbers who refused and who were ineligible for vaccination were smaller than expected.

At present we expect that the first analyses of protective effects of the trial vaccines will be carried out in 1995. Until then the trial population will be followed by a combination of 'enhanced passive surveillance' (individuals are examined by LCAs for signs of leprosy whenever they have contact with health services in the district, be it as patients, guardians of patients, or as attenders of ante-natal or under-5s clinics) and sample surveys in high incidence areas and in areas far from any health service facility. Approximately 50% of the trial population had been re-examined by one or other of these methods by late 1992.

Acknowledgements

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All the KPT staff worked hard, often away from their families for many weeks, to accomplish the intake phase of the trial.

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L'étude de prévention de Karonga: une étude de vaccin contre la lèpre et la tuberculose en Malawi du nord. I. Méthodes adoptées pour la phase de vaccination

J. M. Ponnighaus, P. E. M. Fine, L. Bliss, P. J. K. Gruer, B. Kapira-Mwamondwe, E. Msosa, R. J. W. Rees, D. Clayton, M. C. Pike, J. A. C. Sterne et S. M. Oxborrow

Résumé Nous décrivons en détail les méthodes adoptées pour l'étude de prévention de Karonga, une étude à double aveugle de vaccin contre la lèpre et la tuberculose dans le district de Karonga en Malawi du nord. Au cours d'une enquête porte-à-porte recouvrant la population totale, qui a commencé en novembre 1985 et s'est terminée en août 1989, 121.008 personnes (57.892 personnes de sexe masculin et 63.116 de sexe féminin) ont été vaccinées. 5835 autres personnes ont refusé de se faire vacciner et 5757 autres n'étaient pas admissibles à la vaccination, 2652 d'entre elles parce qu'elles avaient des antécédents de signes de lèpre ou parce qu'elles étaient présumées atteintes de lèpre précoce. Un total de 66.145 personnes chez lesquelles aucun signe apparent de BCG antérieur, n'a été détecté, one été vaccinées comme suit: BCG, BCG $+ 5 \times 10^7$ de *Mycobacterium leprae* tués, ou BCG $+ 6 \times 10^8$ de *M. leprae* tués; 54.863 personnes qui présentaient une cicatrice typique ou douteuse de BCG ont été vaccinées au placebo, au BCG ou (à partir du milieu de l'année 1987) au BCG $+ 6 \times 10^8$ de *M. leprae* tués. Les effets secondaires n'ont pas été recherchés systématiquement mais quatre personnes signalèrent d'elle-mêmes des abcès glandulaires, neuf personnes de grands ulcères post-vaccination (> 25 mm de diamètre) et deux, des ulcères qui ont duré plus d'un an.

Les foiles de BCG recueillies sur le terrain dans des réfrigérateurs à pétrole, avaient des concentrations satisfaisantes de BCG viable pendant toute la durée de l'étude.

Les résultats des tests cutanés post-vaccination (RT23 et l'antigène soluble du *M. leprae*) et les taux d'ulcères post-vaccination indiquent que seules quelques erreurs ont été faites sur le terrain en notant le code des vaccins.

Ensayo preventivo de Karonga: ensayo de vacuna contra la lepra y la tuberculosis en Malawi del Norte. I. Métodos de la fase de vacunación

J. M. Pönnighaus, P. E. M. Fine, L. Bliss, J. K. Gruer, B. Kapira-Mwamondwe, E. Msosa, R. J. W. Rees, D. Clayton, M. C. Pike, J. A. C. Sterne y S. M. Oxborrow

Resumen Se describen en detalle los métodos del Ensayo de Prevensión de Karonga, un ensayo doble ciego de vacuna contra la lepra y la tuberculosis en el Distrito de Karonga, Malawi del Norte. Durante una campaña de la población total, casa por casa, que duró desde noviembre de 1985 hasta agosto de 1989, se vacunó a 121.008 personas (57.892 hombres y 63.116 mujeres). Otras 5.852 personas se negaron a ser vacunadas, y 5.757 no resultaron aptas para la vacunación, de las cuales, 2.652 tenían antecedentes o señales de lepra, o porque se sospechaba estaban en las etapas tempranas de la enfermedad. Un total de 66.145 individuos sin evidencia de previa vacunación BCG recibieron una de las siguientes vacunas: BCG, BCG $+ 5 \times 10^7$ *Mycobacterium leprae* eliminados, o BCG $+ 6 \times 10^8$ *M. leprae* eliminados; 54.863 individuos que presentaron una típica o dudosa cicatriz de BCG recibieron placebo, o BCG, o (desde mediados de 1987 en adelante) BCG $+ 6 \times 10^8$ *M. leprae* eliminados. No se buscaron efectos adversos en forma sistemática. pero cuatro individuos se presentaron para denunciar abscesos, nueve individuos denunciaron grandes úlceras posteriores a la vacunación (> 25 mm de diámetro), y dos denunciaron úlceras que perduraron más de un año. Las ampollas de BCG recogidas de los refrigeradores de parafina en campo mostraron concentraciones satisfactorias de BCG viables durante todo el ensayo. Los resultados de las pruebas cutáneas posteriores a la vacunación (RT23 y antígeno soluble de *M. leprae*) y los índices de úlceras posteriores a la vacunación indican que se efectuaron pocos errores en campo al registrar los códigos de las vacunas.

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Nerve abscess in leprosy: a retrospective study

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Summary Nerve abscesses occur, both in tuberculoid and lepromatous leprosy. We studied 20 patients who had undergone surgery for nerve abscess in mixed peripheral and cutaneous nerves. Details of these cases and the controversial question as to how long the question PB regimen should be continued are discussed.

Introduction

Leprosy is a disease of the skin and nerves, and the involvement of both peripheral and cutaneous nerves is often found in leprosy. Schwann cells act as a reservoir of *Mycobacterium leprae*. The pathology is different in tuberculoid and lepromatous leprosy. In tuberculoid leprosy there is a cell-mediated immunological reaction that causes a granuloma formation which in some cases may liquify and develop into an abscess. In lepromatous leprosy abscesses may be due to an antigen–antibody reaction, e.g. an ENL reaction, exacerbation of lepromatous lesion, necrosis of lepromatous granuloma or, perhaps, an iatrogenic reaction as a complication of perineural or intraneural injection.[1] Multiple nerve abscesses are common in cases of lepromatous leprosy[1,2,3,4,5,6,7] but not in tuberculoid leprosy.

Material and methods

Between January 1987 and December 1990, we carried out a decompression of the nerves of 490 patients who were suffering from early paralysis, painful neuritis and nerve abscesses. (Of these, 20 patients underwent surgery for nerve abscess.) These cases were analysed for the type of leprosy, the duration of the disease, the duration of treatment, which nerve was involved, their bacteriological index, and the assessment of sensory and motor paralysis. After the surgical evacuation of pus and the decompression of nerve, the

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pus was stained for AFB, culture, and sensitivity for pathogens was also carried out. Epineurium, along with caseation and some nerve tissue, was sent for histopathological examinations. We carried out a periodic assessment on any recovery of motor and sensory function.

The surgery involved epineurotomy and the evacuation of abscess from all nerves. Medical epicondylectomy was performed in the cases of ulnar nerve abscess and a carpal tunnel decompression for median nerve lesions.

Observation and analysis

In all, 20 patients, whose average age was 21·3 years (range 9–55 years) underwent an evacuation of nerve abscesses—18 were male and 2 female. The patients had on average suffered for 1·8 years (minimum 2 months, maximum 6 years); 18 had already been on a specific treatment for between 1 month and 2 years—7 were tuberculoid, 11 BT, 1 BL, and 1 LL. The skin smear was positive in only 2 cases, while the abscess material on staining showed AFB in 7 cases (including the 2 cases above). The nerves involved were 17 ulnar, 5 median, 1 lateral popliteal, the others being cutaneous nerves. The duration of abscess varied from 1 to 6 months. Culture and sensitivity of the abscess material did not reveal any pathogenic organisms. All the trunk nerves involved had some functional deficit.

OPERATIVE FINDINGS

In cases of tuberculoid and BT leprosy the pus was thick, the nerve bundles were swollen, and some were granulomatous. There was multiple nerve involvement in BL and LL cases, and the pus was thin. Tiny abscesses were found in many nerve bundles. The epineurium was thick and adhered to surrounding tissues and perineurium. It was difficult to separate the epineurium from nerve bundles.

Discussion

Nerve abscess is often seen in leprosy (Figures 1 and 2). It is more common in the tuberculoid spectrum, especially in borderline tuberculoid cases, probably due to some exaggerated delayed hypersensitivity reactions. In all, 18 patients developed nerve abscess after MDT or DDS monotherapy. Any reactions and relapses that occur during or after treatment can result in nerve damage.[8,9,10] Katoch *et al.*[9] reported a late reaction in 9% and a relapse in 13% of their cases. Pavitran[10] has also reported relapses after PB regimen (12%).

Even though nerve abscess is rarely seen in the lepromatous spectrum of the disease, many have reported nerve abscess in lepromatous leprosy. In the 2 cases reported here, both ulnar and median nerves were involved on both sides. The patients had an acute exacerbation of ENL reactions, after which they developed pain, tenderness and swelling in the nerves. The pus was thin and contained AFB and pus cells. A skin smear of the patients was positive and their BI was 2·3 and 1·5. Abscess formation was caused by an acute exacerbation of ENL lesion in the nerves.

In 5 of 11 BT cases the pus was bacteriologically positive for AFB (1+). The skin

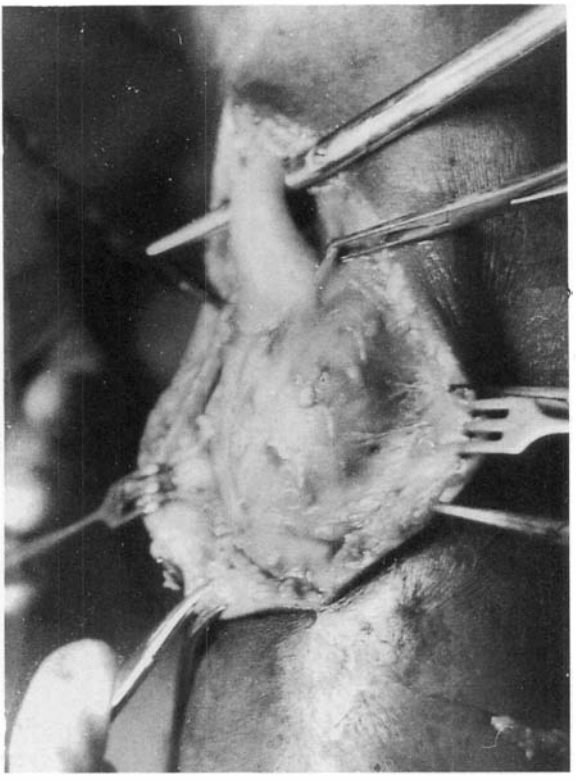

Figure 1. Photograph of an abscess in a lateral popliteal nerve.

smear was negative and skin biopsy from the patch showed a typical borderline tuberculoid type of leprosy with epithelioid cells, some lymphocytes, but no AFB were seen. Therefore, there was a discrepancy in the bacillary content of the nerve and the skin. Palande,[11] Sreenivasan *et al.*[1] and Periera *et al.*[12] have reported a discrepancy of bacterial presence in the skin and nerve lesions.

Periera *et al.*[12] carried out some electron-microscopic studies on treated BT cases and revealed the presence of bacilli and the proliferation of Schwann cells—some were solid bacilli. Pavitran[10] reported a relapse rate of 12% (3/25) after a PB regimen. Kar[13] reported that 35% were clinically active, and 47% histologically active after 6 months of MDT in paucibacillary leprosy. Katoch *et al.*[9] reported that 13% relapsed in paucibacillary cases after MDT. He also reported that the relapse rate fell in cases where the treatment was continued for more than 6 months. This calls attention to the controversial question of insufficient PB regimen in some BT cases. They have to receive treatment for more than 6 months or be treated as MB cases (WHO regimen). This question needs further evaluation and investigation.

Calcification can occur in caseation and necrotic material in tuberculoid and borderline tuberculoid leprosy, as a result of deposition of calcium in the dying, dead and chronically inflamed tissues. Malaviya *et al.*[14] reported 3 cases of calcification in nerves. Ramanujam *et al.*,[15] Jopling[16] and Ellis,[17] reported some calcification in the nerves. In the majority of the reports, the ulnar was the most commonly affected nerve. White chalky

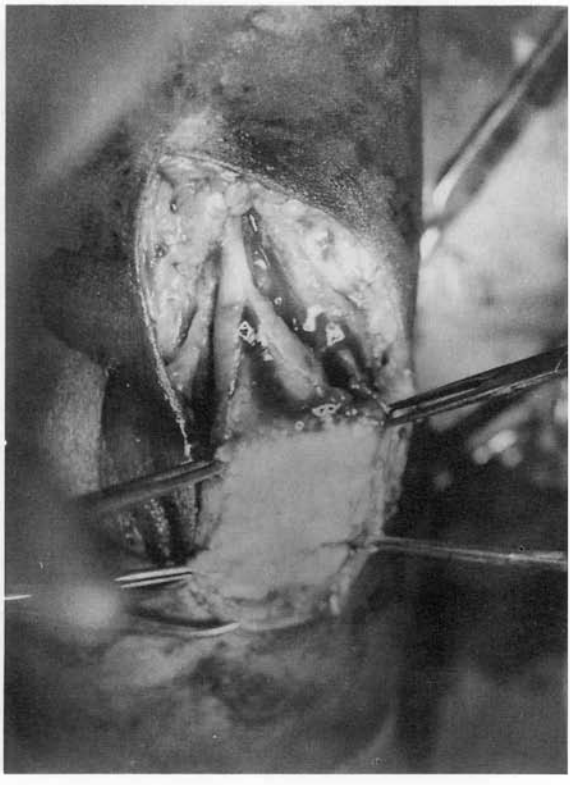

Figure 2. Photograph of an abscess which has been opened. Pus and granulation is seen.

material was found on exploration of the nerves. In our series the ulnar nerve that was thickened and painful was found to contain inspissated pus, chalky material and also a calcified stone, which was removed.

Conclusion

We found 29 nerve abscesses in 20 leprosy patients. Abscess was found in more than 1 nerve in 2 cases of lepromatous and in 3 cases of borderline tuberculoid leprosy. There was a discrepancy in the bacteriological index of the skin and nerve in 5 borderline tuberculoid cases of 11. This highlights the controversial question as to whether to continue the PB regimen for more than 6 months or whether the patients should receive treatment as MB cases (WHO regimen) to prevent any relapses. Calcification of the nerve was seen in 1 borderline tuberculoid leprosy patient, from whom a calcified stone was removed.

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Lepr Rev (1993) **64,** 357–61

Les abcès nerveux dans la lèpre: une étude rétrospective

M. K. Siddalingaswamy et K. S. Rao

Résumé Les abcès nerveux ont lieu dans la lèpre tant sous sa forme tuberculoïde que lépromateuse. 20 sujets ont subi une intervention chirurgicale pour abcès nerveux des nerfs périphériques et cutanés mixtes. Nous décrivons ces cas et parlons de la question discutable de la durée du régime thérapeutique PB.

Abscesos nerviosos en la lepra: un estudio retrospectivo

M. K. Siddalingaswamy y K. S. Rao

Resumen No hay duda de que se producen abscesos nerviosos en la enfermedad de Hansens, tanto en la lepra tuberculoide como en la lepromatosa. Veinte pacientes fueron intervenidos quirúrgicamente debido a abscesos nerviosos en nervios periféricos mixtos y cutáneos. Se discuten los detalles de estos casos y la cuestión debatible de la duración del régimen PB.

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Teaching Materials and Services; News and Notes

CBM/LEPRA Ophthalmic Course, Karigiri, India 1993

The Eighth Annual Five-day Ophthalmic Teaching Module was held at the Schieffelin Leprosy Research and Training Centre, Karigiri from 1 to 6 March 1993. This course, which was again sponsored jointly by the Christoffel Blindenmission and LEPRA, was designed to give instruction to leprologists on the detection, prevention and management of the ocular complications of leprosy by means of a series of lectures, clinical and surgical demonstrations, videos and slide-tapes.

Teaching included presentations on basic anatomy, physiology and pathology of the eye with special emphasis on leprosy: in addition there were lectures on the clinical signs and management of lagophthalmos, corneal ulcers, intra-ocular inflammation and infiltrative lesions, together with discussions on 'high risk eyes', ocular manifestations of relapsed disease, rehabilitation and the global aspects of blindness in leprosy.

The course, which was attended by 15 participants from India, Bangladesh, Nepal and the U.K., was run by Dr Margaret Brand of The Leprosy Mission and Mr Timothy ffytche from St Thomas's Hospital, London, together with Dr Ebenezer Daniel and Dr Mary Jacob of Karigiri.

The Director and staff of Karigiri and The Leprosy Mission are to be congratulated on their continued support for this important and popular contribution to teaching.

Report on the visit of Mr T. J. ffytche FRCS to the SLRTC Karigiri, 1–6 March 1993

I have just returned from the Schieffelin Leprosy Research and Training Centre, Karigiri, having assisted in the running of the annual ophthalmic course, and I would again like to express my sincerest thanks to LEPRA for their financial support.

This was the 8th annual CBM/LEPRA ophthalmic course held at Karigiri and it continues to be an established part of the training programme in the Centre, taking place at the end of the 6-week general course. This year there were 15 participants of whom 13 were leprologists working in India, Bangladesh and Nepal and 2 were ophthalmologists, one from TLM in Delhi and 1 from England about to take up an appointment working in ocular leprosy for TLM in West Bengal. In all 1 participant was sponsored by LEPRA and 11 by TLM, 2 doctors from Bangladesh were supported by the Damien Foundation and the Dansi Leprosy Mission respectively; 6 leprologists who were due to attend the course failed to turn up, and of these 5 were sponsored by LEPRA.

The length of the course was again $5\frac{1}{2}$ days and the structure was similar to that of previous years with a total of 13 lectures of 1 hour each covering the following topics:

Basic anatomy and physiology;
Ocular pathology in leprosy;
Clinical signs, management and surgery of lagophthalmos;
Infiltrative ocular lesions in leprosy;
Diagnosis and management of the red eye;
Ocular manifestations of relapsed disease;
The 'high risk' eye;
Ocular manifestations of relapsed disease;
Blindness in leprosy and its global considerations.

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In addition there were daily clinical demonstrations where participants were instructed in methods of examination and shown simple surgical and therapeutic procedures, together with teaching videos and slide-tape presentations. Participants were given a multiple choice exaination paper at the beginning and at the end of the week to assess their progress, and they were asked to evaluate the completed course by filling in a short proforma.

The teaching faculty consisted of Dr Margaret Brand, Mr Timothy ffytche, Dr Ebenezer Daniel, Dr Mary Jacob and Mr Prem Kumar, and the course was organized by the staff of Karigiri under the directorship of Dr J. A. Ponniah.

The group, which consisted of doctors from India, Bangladesh, Nepal and the U.K., proved to be an excellent mixture. This was the most extensive of the 8 courses that have been held, and this meant that participants were less able to have 'hands on' experience in surgery, and individual tuition in clinical examination was less easy to organize; however, the presence of 2 ophthalmologists, Dr Thompson and Dr Silas, was most helpful as they were able to assist in the clinical teaching.

It was very gratifying to see that several members of the staff were able to attend the morning lectures in addition to the course participants, as this had been recommended in previous reports.

The week ended with a formal ceremony in which certificates were presented to participants on the ophthalmic, physiotherapy and workshop courses, with a valedictory address given by Dr Ernest Fritschi, a previous Director. This event was highly successful and it is hoped that it will be repeated in future years.

We would again like to pay a special tribute to Dr Ebenezer Daniel, the resident ophthalmologist to Karigiri, whose enthusiasm and teaching skills greatly contributed to the success of the course.

LEPRA had sponsored 6 participants this year, although 5 were unable to attend. The reasons for this have yet to be ascertained, although from a practical point of view the increased numbers would have made the group too large and therefore less effective for clinical teaching. The length of the course is now established at $5\frac{1}{2}$ days, and this has the advantage of allowing the participants to spend extra time in the out-patient department, so that by the end of the week most felt confident that they would deal with the sort of ocular problems that leprosy produces, and had had sufficient experience in eye examinations to be able to train their paramedical workers. This represents one of the fundamental aims of the course.

Recommendations for 1994:

On the basis of the experience with the 1993 course, I would like to repeat some of the recommendations that have been made previously:

1. That the CBM/LEPRA ophthalmic course should continue to be held annually at the end of the 6-week January general course, and that the length of the course should be $5\frac{1}{2}$ days (Monday–Saturday am).
2. That the 2 main invited speakers should continue to be supported by CBM and LEPRA (Dr Brand or her successor by CBM; Mr ffytche or his successor by LEPRA), and that these 2 charities should make an appropriate contribution towards the administrative costs incurred by the Centre in the running of the course.
3. That the participants should be mainly leprologists *not* ophthalmologists and that the numbers accepted for the course should be not less than 12 and not more than 16.
4. That LEPRA should be allowed the option of sponsoring at least 3 leprologists to attend the course, and that these individuals should be selected before September 1993 by LEPRA (India) if possible, and the information about them relayed to the Director at Karigiri with copies sent to LEPRA in London.
5. That the course be advertised at an early stage through leprosy journals and health authorities as well as through charities such as CBM, TLM and LEPRA.
6. That the structure of the course remains basically the same as before, and that the morning lectures should be open to anyone working in Karigiri or attached to the Centre. The afternoon sessions of clinical and surgical teaching should be restricted to course members only.

I would like to conclude by thanking LEPRA again for their continued support, and by expressing my sincerest gratitude to Dr Ponniah and all the staff at the Schieffelin Leprosy Research and Training Centre for their kindness and hospitality during my short stay in Karigiri.

T. J. ffytche FRCS, FCOphth
21 March 1993

The Panafrican Institute of Community Health (IPASC)

IPASC aims to provide the quality of care in NGO and church-related health programmes in Africa. It is situated in the Rural Health Zone of Nyankunde Hospital, Zaire, and has academic support and collaboration of the Liverpool School of Tropical Medicine.

The activities of IPASC include:

a The **training** of health programme leaders, supporting them on their return to their work through follow-up visits, and working in collaboration with the communities and NGO/church-related programme leaders. IPASC courses are at various levels, using a participatory learning approach and including considerable field experience.

b **Research** into the causes of the poor quality of care, and the lack of sustainability, particularly at the peripheral level, so that guidelines may be established for the formulation of health policies and for the organization and management of health programmes.

c Developing a **consultancy service** in community health, including making available IPASC's training resources for use in other countries.

For further details: Institut Panafricain de Santé Communautaire, PO Box 21285, Nairobi, Kenya, Fax number: AIMSERVE (2542) 501651.

PAHO: Eradication plan for the elimination of leprosy from the Americas

The following is extracted from the *Proceedings of the XLIV Meeting* of the Pan-American Health Organisation, September 1992, held in Washington, DC, USA:

'In the consideration of this item at the 109th Meeting of the Executive Committee, the point was made that, because of the stigma associated with leprosy and its potential to produce serious disability, the Region's experts consider that the disease is a much greater public health problem than the prevalence figures alone would indicate. There are approximately 300,000 cases of leprosy in the Region, the case rate being 4·2 per 10,000 population. In 1991, 30,000 new cases were detected. The disease is not uniformly distributed in the Region or in the countries; 80% of all cases are found in Brazil, where the majority of new cases are also detected. Colombia, Mexico, and Venezuela also have more than 10,000 cases each. Leprosy can be considered a public health problem in 21 of the countries.

At present—through multidrug therapy (MDT) with dapsone and rifampicin for paucibacillary cases and with dapsone, clofazimine, and rifampicin for multibacillary cases—it is considered possible to attain the goal of elimination, i.e., less than one case of leprosy per 10,000 population. Unfortunately, MDT has not been as widely used in the Americas as elsewhere in the world. The plan for the elimination of leprosy in the Region emphasizes the need for early detection of cases and increased MDT coverage, and proposes that the required actions be carried out through the local health systems.

In the Committee's discussion of this item, it was mentioned that technical assistance and advisory services should be concentrated in the countries where incidence and prevalence of the disease are high. It was pointed out that there are several potential obstacles to the goal of elimination—for example, the difficulty of determining incidence and detecting cases early, and the possibility that, in countries where leprosy is not very prevalent, the integration of leprosy prevention and control activities into other programs might interfere with accomplishment of the program's goal of reducing prevalence rates. Concern was also expressed about the cost of multidrug therapy.'

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XIV International Leprosy Congress Orlando, Florida, USA 29 August–4 September 1993

Editorial

The XIV International Leprosy Congress was organized under the sponsorship of the International Leprosy Association, in collaboration with the International Federation of Antileprosy Associations and the World Health Organization, and was held in the Buena Vista Palace Hotel, Orlando, Florida, USA from 29 August to 4 September. Presentations to the Congress consisted of state-of-the-art lectures, oral and poster presentations of original papers and a variety of exhibitions. In addition, the Congress presented an extensive short course teaching programme consisting of 20 courses on such diverse topics as communication skills for health workers, vocational rehabilitation, the cellular immunology of leprosy and PC Basic. These courses were extremely well attended, frequently being oversubscribed, and were a very successful addition to the Congress programme.

The state-of-the-art lectures covered 'The microbiology of *Mycobacterium leprae*' (Dr S. Cole, France, and Dr P. Brennan, USA); 'Immunology: lessons from and for leprosy' (Dr T. Ottenhoff, the Netherlands); 'Chemotherapy' (Dr J. Grosset, France); 'The elimination of leprosy' (Dr S. K. Noordeen, Switzerland); 'Not by chemotherapy alone' (Dr H. Srinivasan, India); and 'Sustainability and cost-effectiveness of leprosy control under low prevalence conditions' (Dr P. Feenstra, the Netherlands). Drs Brennan and Cole reviewed the amazing progress that has been made in recent years on our understanding of the structural chemistry and genetic organisation of *M. leprae*, while Dr Ottenhoff led us on a tour through the immune system, pointing out the conflict between its role in protection against disease on one hand, and its involvement in pathological mechanisms on the other. Dr Noordeen outlined the progress made to-date with the implementation of MDT, explained the basis of WHO's elimination target and detailed the resources required to ensure that these targets were achieved. Professor Grosset explained the rationale for the MDT regimen design, and the position with regard to the development of new antileprosy drugs and how they might be incorporated into 'second' generation regimens aimed at reinforcing the MDT campaign. Dr Srinivasan stressed the importance of planning the transfer of rehabilitation and re-enablement techniques, so that these will not disappear along with the control programmes when disease prevalence falls. Finally, Dr Feenstra also took up the theme of strategic planning in the face of low prevalence, describing models for the organization of control programmes when it is no longer practical to retain a vertical programme structure.

There is no doubt that the most lively area of debate involved the WHO's campaign for 'elimination of leprosy as a public health problem by the near 2000', a topic which

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could be heard in the coffee rooms, bars and lounge areas of the Conference hotel. In his key note address to the Congress, Dr Paul Brand had given an extremely entertaining and erudite warning of the dangers of predicting elimination of leprosy, reminding us of the predictions which accompanied the introduction of sulphone treatment and drawing comparisons with other infectious diseases which were once thought to be on the verge of eradication. He questioned the selection of the prevalence figure of 1 case per 10,000 as being the target below which the disease would be declared as being no longer a public health problem. Dr Noordeen, in his state-of-the-art speech, acknowledged that there would still be much work to be done even if the 'elimination' target was reached by the year 2000 and that research in such areas as vaccine development, immunology, microbiology and clinical fields should be continued. Most delegates were buoyed by the positive and optimistic message contained in Dr Noordeen's address, while at the same time being conscious of the difficulties ahead and the dangers that lurked if the optimism was replaced by complacency.

In the laboratory sciences, the most impressive progress was seen in our understanding of the basic biology of the mycobacteria, particularly *M. leprae*. The genome project, outlined by Dr Stewart Cole in his state-of-the-art lecture, along with the application of modern molecular techniques presented by many scientists in original papers, are advancing our knowledge in quantum leaps. In addition, to our understanding of the genetic organization of *M. leprae*, clues to the basis of its pathogenicity are being revealed while molecular techniques are being used for more immediate application such as the rapid detection of drug-resistance.

Our understanding of the immunology of leprosy is also keeping pace with basic advances in the field of immunology. At the previous Congress, held in 1988, the use of molecular techniques to identify antigens of *M. leprae* was the major talking point. Now interest is focused much more on trying to understand the cellular and molecular networks which determine the outcome of infection and immunization with mycobacteria. In this respect leprosy is not only seen as an important infection in its own right, but is used as a 'paradigm' for understanding the basis of immunity to intracellular infections in general. Although there has been much progress in understanding such interactions, the 'holy grail' of leprosy immunology—the basis for lack of responsiveness in lepromatous leprosy patients—still appears to be as elusive as ever.

There was a general recognition in many presentations of the importance of reversal reactions. Some of the molecular approaches which have been introduced in recent years are now being targeted towards understanding the immunological basis of immunopathology and nerve damage in leprosy. A recurring theme in several presentations was the possible role of 'molecular mimicry', in which the body's defence mechanisms aimed at bacterial antigens in fact recognized similar structure in the host's own tissue. As with protective immunity, the cytokine network during immunopathological responses is also a target for much research, and there was much discussion and debate surrounding the reports of novel methods for inducing nerve regeneration. Since the problems of reversal reactions, nerve damage and immunopathology will be with us for many decades it is encouraging to see research in these areas at last being recognized as a priority.

There were excellent contributions from all over the world covering a broad range of clinical and social aspects of leprosy. It is essential that the information obtained from well-conducted studies, and the motivational qualities of such work should be maintained as leprosy control enters a new era.

The overriding impression of the Congress was one of being at a turning point. The emphasis is shifting; while the development of new drug regimens, research into vaccines, etc. is continuing, there was a feeling that these are no longer the key issues. The reduction in prevalence in most leprosy-endemic areas will bring new challenges to everyone involved in leprosy research. How can leprosy control be incorporated into primary health care strategies when vertical programmes are no longer cost-effective? Can the implementation of MDT be accelerated and can new, rapidly bactericidal drugs be incorporated into this process? Can we increase our understanding of nerve damage so that novel approaches to reducing disability can be developed? The dividing line between optimism and complacency, between triumph and failure is a fine one. By the time of the XV Congress in Beijing in 1998, we will be well down the road to knowing how these challenges are being met and to which side of that line we are heading.

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XIVth INTERNATIONAL LEPROSY CONGRESS

Workshops

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Workshop 1 Microbiology

Chair: F. Portaels
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The progress made on various aspects was reviewed as follows:

Purification of *Mycobacterium leprae*

Armadillo-derived *M. leprae* continue to be used for various biochemical, structural and antigenic studies. A modified hybrid protocol (containing alkaline treatment and 30% Percoll density gradient—1/79 and 1/77) particularly aiming to remove pigmented host material and useful for the purification of *M. leprae* armadillo liver has been developed. In addition, an assay based on the estimation of arabinose content to check the purity (mycobacterial content) of bacilli after purification has been described. However, it was felt that the effect of gamma irradiation on various viability markers/tests and integrity of DNA (which may influence the PCR signals) is not fully understood. Also, there are no objective techniques to monitor the contamination of purified *M. leprae* with soluble host material and other mycobacteria.

Cultivation

Attempts to grow *M. leprae* in modified conventional/earlier described media (7H9, Dubos and DH) as well as unconventional (simple media for chemoautotrophs) have been described. Chemoautrophic nocardioform (CAN) organisms from *M. leprae*-infected tissues have been repeatedly isolated, subcultured and shown to resemble *M. leprae* by enzymatic, chemical (mycolates and PGL), antigenic (lepromin), 36 kD PCR and pathogenic (mouse mutation) criteria. Further, coccoid, mycelial, cystic and spore-like forms (nocardioform arthrospores and blastospores) in the growth cycle of leprosy bacillus have been postulated in the recent reports. These investigations need to be further pursued and isolates taxonomically fully characterized. There were presentations which demonstrate that PCR could be a taxonomic tool. However, keeping in mind the danger of 'carry over', the need to try other genomic markers such as DNA/DNA, DNA/RNA hybridization (overall and using specific probes) as well as RFLP analysis was suggested, and the necessity of having confirmed 'pure' and 'viable' organisms in the cultivation attempts was emphasized.

Physiology/Biochemistry

Studies to investigate the physiology of *M. leprae* by biochemical and molecular approaches were reported. Enochelins and ferritins have been identified in *M. leprae*. Further studies have shown that while purine biosynthesis is undetectable in *M. leprae*, this organism is capable of pyrimidine biosynthesis and scavenging. *M. leprae* has been shown to have the capacity to utilize/hydrolyse several host lipids. Acetate has not been found to be incorporated in *M. leprae*, possibly because of the absence of phosphotransacylase: fatty acid syntheses have been detected, though at low activity. In contrast, fatty acid elongases were readily detectable in *M. leprae*. Phospholipids have been observed to be hydrolysed by *M. leprae* but this does not obviously damage the host membranes. The organisms have been reported to be capable of utilizing acylglycerols (using a model but not so far using any natural subtrates). It is not yet known if *M. leprae* can use sphingolipids, which are the major neural lipids. It has been suggested that the possibility of drugs that combat PGL and LAM biosynthesis, and an immunotherapy to block the entry and prevent persistence in Schwann cells and macrophages should be investigated.

Further proteins of *M. leprae* have been identified and, for many, their functions deduced. Their genes have been cloned and sequenced to varying extents. The important ones are: 10 kD (gro ES); LSR; 28 kD (SOD); 28 kD (IRG); major cell wall proteins (histone derived from host tissue); a major membrane protein (homology with bacterioferritin) and 6 'less abundant' proteins (2 possibly virulence factors—showing homology with alkyl hydroperoxide reductase and a thiosulphate sulphurtransferase, a 3rd with LT/LIC ribosomal protein homologue). Some of these proteins may have a role in the response of organisms to oxidative/other stresses and also in possible virulence.

Several recent studies have focused on the physiochemical factors important in the growth of *M. leprae*. Using bioluminescence, ^{3}H thymidine uptake and other parameters, several physiochemical factors like nutrients—gelatin, pyruvate, malate, silicone, fossil-fuel derivatives, purines, urea, glycerol, asparagine, etc., and physical conditions such as lower pH 6–6·5, temperatures of 3–30°C, and lower oxygen levels have been identified as possibly relevant for the *in vitro* growth of *M. leprae*. These factors and appropriate procedures like the processing of specimens, the addition of large quantities of lipids as cyclodertrin complex (sphingolipids, palmitic acid), etc., were suggested as methods to improve the ATP synthesis/possible growth in any future studies. The need to analyse experiences about other difficult-to-grow mycobacteria to identify critical factors that are possibly important for *M. leprae* was emphasized.

Sequencing of genes of *M. leprae*

Information detailing several gene sequences of *M. leprae* has become available, and on the basis of published data about ribosomal RNA genes, the leprosy bacillus has been shown to belong to the slow-growing mycobacterial cluster. In addition, the genome sequencing project on *M. leprae* has been progressing and about 15% genome has already been sequenced. This information has been reported to be complementary to data that is emerging about several genes coding for structural proteins of *M. leprae*. If the present funding levels are maintained, the entire genome of *M. leprae* could be sequenced in about 3 years.

Gene probes/amplification methods

Several gene probes and gene amplification techniques to detect *M. leprae* gene sequences have been developed and there are some others still under development. Among the important gene amplification techniques are those targeting 18 kD, 36 kD, 65 kD, ribosomal RNA and repetitive DNA sequences. Rapid genetic techniques are also being developed to detect mutations conferring resistance to rifampicin in *M. leprae*. Data about the application of different PCR assays in the clinical specimens as well as standard specimens (including IMMLEP trials) shows that the techniques are practicable. These assays appear to be generally sensitive for MB cases whereas sensitivity is about 50% for smear negative PB cases. The optimum methodology for specimen collection, storage, extraction and criteria of positivity (whether by EB/autoradiography) need to be further refined in greater detail to establish their applicability in clinical diagnosis and epidemiology.

***In vitro* estimation of viability**

In the absence of acceptable definition of 'cultivable unit' of *M. leprae*, all other criteria continue to be indirect. Data discussed showed that *in vitro* methods are proving to be useful for screening agents without encountering the problems of pharmacokinetics of mice. In addition to earlier discussed methods like MI, electronmicroscopy, FDA-EB staining, metabolite/substrate uptake assays, various macrophage assays, newer or modified techniques based on biochemical, bioluminescent and molecular approaches have been described as alternatives to mouse footpads. Data on the application of FDA-EB staining, bacillary ATP measurement, Na+/K+ ratio measurement by LAMMA, radiorespirometry (BACTEC/Buddemeyer systems) and gene probe/amplification (limited dilution PCR, different quantitative PCRs, ribosomal RNA based systems) show that several approaches can be useful for monitoring the responses to chemotherapy, drug screening and *in vitro* measurements of metabolic status of *M. leprae*. Since these methods assess different aspects of viability, even more than one method may be useful/necessary for a particular purpose. Using radiorespirometry, newer compounds active against *M. leprae* have been reported which have been shown to be promising in clinical trials later. Also, LAMMA, other uptake assays, and ATP decay assays have been shown to be useful for drug screening. Follow-up studies are required to adapt and assess these methods for their ultimate clinical or laboratory application.

Possible environmental sources of *M. leprae*

There appear to be relationships between the distribution of fossil fuels and endemicity of leprosy which should be epidemiologically investigated. New techniques will provide the necessary tools to further investigate the possibility of an environmental reservoir for *M. leprae*.

Areas of future research

(a) When considering the experience with tuberculosis, where, in addition to cultivation, rapid and alternative methods of research are also required, it is felt that parallel

efforts on cultivation, as well as on the development and application of some alternate methods for viability, detection and identification of the characteristics of *M. leprae* in patients should be researched, and therefore environmental and laboratory studies should be continued.

(b) Molecular approaches to fill the criteria gaps in the physiology/biochemistry, structural aspects, drug resistance and virulence factors should be investigated in future studies.

(c) Studies on the development and application of gene probes/gene amplification methods to diagnose and investigate the epidemiology of disease should be given special attention.

(d) In addition to already known taxonomic characteristics, newer molecular tools should be used to establish the identity of any 'cultivable' form of *M. leprae.*

(e) The need for bacillus and its component parts for various purposes remains high and it is still important to find answers to several questions (for example, even when the genome sequencing project is complete, it will still be necessary to know which genes are expressed). To meet these research demands, 'pure' *M. leprae* should be provided in the future and the supplies from armadillos/nude mice should continue to be a priority.

Workshop 2 Immunology

Chair: J. D. Watson

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Towards The Year 2000

Throughout history no infectious disease has been eradicated solely by chemotherapy, and it is unlikely that leprosy will be the first. Perhaps the greatest contribution research on immunology of leprosy has made has been its role at the cutting edge of basic immunology. Understanding protective immunity and immunopathology in leprosy has ramifications that stretch in the future, far beyond leprosy itself. It is essential to maintain current research momentum to understand the host–pathogen interactions in leprosy in order to develop the diagnostic tools and new immune-based therapies that are still needed in the treatment of disease. By the year 2000, many people will still be infected with *M. leprae* and the clinical disease will continue to emerge far beyond the turn of the century.

Immunology of the Disease Process

The aim of the workshop was to discuss current research on the immunology of leprosy and prepare a summary of research progress in the past 5 years and indicate areas that will continue to dominate future research directions.

Tuberculoid leprosy patients show a strong T cell-mediated immunity to *M. leprae* which is also seen in healthy contacts. Lepromatous leprosy patients have extremely high bacillary loads and widely disseminated lesions, with a striking absence of specific T cell-mediated immunity to *M. leprae*. While T cell-mediated immunity limits multiplication and dissemination of bacilli in tuberculoid leprosy, it does not provide protective immunity in patients and often leads to severe tissue damage. Immunology research seeks to understand the basis of protective immunity and those immune responses involved in tissue pathology such as that seen in nerve damage.

Infection and Disease

To establish infection in the host, *M. leprae* must be taken up by host macrophages, survive and multiply. The development of this relationship between the macrophage and the leprosy bacillus leads ultimately to the clinical spectrum of disease.

In the past 5 years, it has become clear that the successful intracellular multiplication of *M. leprae* leads to a loss of normal macrophage function, impairing the process of antigen-presentation and the subsequent activation of T cells. Within the last 2 years, methods have been developed to identify mycobacterial genes whose expression is induced in the microenvironment of the macrophage. Such gene products may be important in modulating macrophage function, such as intracellular pathways that are designed to inhibit bacterial multiplication. These studies should be extended to determine the genes of *M. leprae* that are induced in Schwann cells, as these may differ from those expressed in macrophages.

Lipoarabinomannans (LAM) purified from *M. leprae* (Lep-LAM) are potent macrophage regulatory factors. Characterization of LAM from other mycobacteria have revealed unique chemical modifications in mannose capping that appear to correlate with function. For example, Ara-LAM from *M. tuberculosis* H37Ra, which is a rapid growing avirulent strain, lacks mannose capping and leads to the induction of levels of the cytokine tumour necrosis factor (TNF) which are 100–1000 fold greater than that induced by Man-LAM from virulent *M. tuberculosis* H37Rv. As the induction of TNF may be central in resistance, Man-LAM may be a tuberculosis virulent factor, and may be important also as a leprosy virulence factor. All LAM species inhibit the production of interferon-gamma (IFN-gamma). In murine macrophages, IFN-gamma induces the intracellular synthesis of nitric oxides which directly inhibit mycobacterial multiplication. Human macrophages are more difficult to understand. The pathway leading to the arginine-dependent induction of nitric oxides has not been identified in human macrophages; however, treatment of lesions in lepromatous leprosy patients with IFN-gamma appears to lead to the elimination of bacilli. The mechanism underlying this response is unknown.

A clear priority is to determine how human macrophages respond to infection with

M. leprae. It is also becoming important to identify differences in tissue-specific populations of macrophages, as well as the kinetics of macrophage turnover.

Protein Antigens of *M. leprae*

In the last 5 years the focus of work has been the definition of the immune repertoire of the host, and the identification of specific antigens of *M. leprae*. Between 15 and 16 different protein antigens have now been identified by antibody reactivity. T cell responses have been characterized to less than half of these proteins and there are few studies reporting direct comparisons, making direct evaluations difficult. In general, individual patients are capable of responding to a broad range of these antigens. There is extensive cross-reactivity of T cell responses to individual proteins between different species of mycobacteria. No single *M. leprae*-specific protein antigen has been defined, although a small number of species-specific T cell epitopes have been recognized. As yet, few proteins have been capable of stimulating protective immunity against *M. leprae* infection in mice. Although certain cell wall fractions, and the 35 kD and 10 kD proteins, may be capable of eliciting partial protection, other events induced at the time of exposure to these antigens may be more important in developing protective immunity.

There are now new methods which can be used to identify protein antigens expressed specifically within macrophages and secreted by mycobacteria. As the *M. leprae* genome will now be completely sequenced, novel techniques are required to make use of the new genetic information that will become available. These may include the definition of T and B cell determinants based on sequence motifs. Once isolated, new proteins must be rigorously purified to avoid contaminants which may confound immunological studies.

As yet, no immune responses to defined antigens of *M. leprae* have been associated with different patterns of immunopathology seen in clinical disease. This may indicate that quantitative rather than qualitative differences in immune responses are important.

Regulation of Immunity

In the past 5 years, emphasis has moved from the concept that there may be specific immunodominant proteins of *M. leprae* that are central to protective immunity, to the realization that the type of response of effector T cells to immunization or infection is likely to be more important for protection. The concept of suppression has become more firmly established but the mechanisms involved and the relationship to lepromatous leprosy remains to be determined. The complexity of the cell types that respond to infection or immunization has increased. These now include NK cells, T cells that express alpha/beta and gamma/delta T cell receptors, and cytotoxic cells in both CD4+ and CD8+ subpopulations. Nonetheless, their role in protection and immunopathology is still unclear. The concept of T_H1- and T_H2-like cells in both CD4+ and CD8+ T cell subpopulations in humans is widely accepted.

Current work is aimed at defining how different types of effector T cells are regulated and determining which cytokines are the primary mediators of immune responses. There is a need to understand the complexity of the cytokine network and how it dictates the outcome of an immune response. The mouse is now used extensively as a model for the genetic deletion of pathways involved in specific immune reactions and these studies are

providing new insight into the regulation of the immune system. Much can now be learned from studying the immunology of tuberculosis and other mycobacterial infections in parallel with leprosy.

Diagnostic Assays

Serodiagnostic assays using *M. leprae*-specific molecules like PGL (NT-P/ND-O-BSA), the 35 kD and the 36 kD proteins, and LAM have been carried out in the past to search for antibodies against these molecules. These studies clearly reveal that not all established cases of leprosy are detectable using these assays. FLA-ABS tests detect a proportion of subjects with subclinical stages of disease. Recent findings using recombinant proteins have detected far less leprosy cases compared to the natural proteins. The finding of antibodies against the cross-reactive 29 kD/33 kD antigens in leprosy sera shows promise; however, their utility in the detection of early cases of leprosy requires further evaluation. Assays for detecting *M. leprae* antigens in leprosy sera have proved to be far less satisfactory compared to the antibody-based assays. It has been pointed out that it would be worthwhile to develop antibody-based assays from slit-skin smears of early lesions of leprosy as a means of increasing the sensitivity of assays.

In reactions, a transient T-cell boost has been observed. Circulating immune complexes (CICs) and antigens like PGL, and 65 kD antigen in the CICs have been demonstrated in a certain number of reactional cases. There has been no association of HLA-DR antigens with any of the types of these reactions. Antibody to LSR2 peptides as well as TNF levels have been suggested as new predictors of ENL and require evaluation.

MLSA (Rees Ag/Convit Ag) and lepromin remain as DTH evoking antigens. The search for new low molecular weight proteins like the 12 kD and 10 kD antigens for use as DTH inducing antigens should be continued and their further evaluation is necessary to prove that these are better evaluators of *M. leprae*-DTH than the existing antigens.

Combinations of *M. leprae* and BCG, and other mycobacterial species-like ICRC, *Mycobacterium w*, have been used as immunotherapeutic agents and have been taken to vaccine trials. The first survey using combinations of *M. leprae* and BCG in protection showed marginal benefits. *M. habana* has been taken as another agent for future vaccine trials.

Research Priorities

These build upon the considerable research achievements of the past 5 years and are divided into basic and applied categories:

BASIC IMMUNOLOGY

(1) Determining how human macrophages respond to infection by *M. leprae*;
(2) defining the cells and mediators that regulate the induction and suppression of effector T cells and antibody responses in immune responses;
(3) continuing the search for new antigens of *M. leprae* and co-ordinating the comparative immunological analyses of standardized recombinant antigen preparations;
(4) using gene knockout technology to investigate cellular, intracellular and cytokine

pathways that combine to provide protective immunity to mycobacterial infections in experimental models;
(5) initiating approaches to the investigation of the immunological basis of nerve damage.

APPLICATION OF BASIC RESEARCH

(1) Develop a superior DTH-evoking antigen which specifically detects *M. leprae* sensitization, and design *in vitro* correlates;
(2) continue to search for the various immunological tests that detect preclinical leprosy, diagnose early leprosy, monitor therapy and detect relapse cases;
(3) emphasize the need for immunological markers for prediction of nerve damage, and for Type I and Type II reactions;
(4) work with microbiologists to find ways of determining the infectivity of *M. leprae* in the population.

Concluding Comments

As an infectious disease, leprosy has plagued mankind for centuries. The co-ordinated efforts of many dedicated individuals from all walks of life have had a dramatic effect upon reducing the prevalence and incidence of disease in the world. The difficulties that result from the length of time it takes to develop the clinical disease following infection and the lack of any sensitive tests that detect preclinical and early stages of the disease impose severe constraints on the efforts to eradicate the disease. The slogan 'eradication of leprosy by 2000 AD' is now beginning to place undue pressure on health and research workers alike. While health workers may begin to place less emphasis on diagnosing early signs of leprosy, to reduce patient lists, research workers are beginning to see the reluctance of funding bodies to continue supporting their activities as leprosy research is no longer a priority. At a time when diseases such as malaria, tuberculosis and AIDS are spreading, the dictum should revert to 'control of leprosy by 2000 AD' rather than eradication. Immunology remains a very real force in the improvement of health for all, and its contribution to leprosy will be substantial.

Workshop 3 Chemotherapy

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S. R. Pattyn
J. Seydel
W. von Ballestrem

Invited but unable to attend:

J. Grosset
M. I. Gunzareth

The Workshop reviewed the progress of multidrug therapy (MDT) in the context of reported side effects and relapse rates. The application of MDT has been accepted world wide over the past decade. More than 3 million leprosy patients have already received WHO and similar MDT and many have been released from control. However, about 50% of the currently-registered patients are not receiving MDT. Therefore, the greatest immediate need is to implement MDT to all registered cases.

In spite of the general acceptance of clofazimine, a drug which has the additional value of reducing the incidence of erythema nodosum leprosum (type II) reactions, its effect of increasing skin pigmentation may result in poor compliance in certain patients and ethnic groups. The alternative, prothionamide, has some gastric intestinal side effects and a dose-related hepatotoxic effect. Other problems encountered include those of geography (so that monthly supervised drug distribution may be difficult because of the terrain), intercurrent disease, inadequate infrastructure and in paucibacillary leprosy (PBL) the difficulty of distinguishing late reversal reaction from bacteriological relapse due to treatment failure. Fortunately, rifampicin resistance remains very rare although its prevention depends upon careful and correct implementation of MDT.

Relapses

It is now over 20 years since the start of the Malta trial and more than 10 years since the introduction of WHO-MDT. Some information on relapse rates in multibacillary leprosy (MBL) is now available, although this is largely based on the original groups of patients, many of whom had received prior, long duration dapsone monotherapy, so that their bacterial loads were often low. In Malta and Paraguay (using rifampicin and isoprodian) and in South India (using WHO-MDT and the similar THELEP regimen), rates have been extraordinarily low and very acceptable. The few relapses detected have occurred 5 or more years after stopping therapy. However, very recently reported studies in Africa, in which varieties of short course regimens were given to previously untreated MBL patients, have resulted in significant relapse rates, some of which are unacceptably high. Moreover, relapse rates were significantly higher in patients with a high BI (5·0 or more) compared with those with a BI of 4·0 or less. Latest results suggest that the rate is probably

unacceptable in a group which received the WHO MBL regimen for a fixed duration of 2 years. Therefore, the Workshop suggested that the fixed-duration regimen may prove to be inadequate in previously-untreated LL patients with a high bacterial load, and counselled caution in the widespread adoption of 2-year fixed duration treatment of WHO MDT until further data are available. It also noted that many relapses are occurring late and, therefore, 5 years' post-treatment follow-up appears to be very inadequate, 8–10 years being the minimum required. In view of a claim that a period of daily rifampicin has some advantages in terms of relapse rates over totally intermittent rifampicin, ongoing analysis of data from long-term follow-up of such regimens is needed.

PBL relapse rates have been acceptably low worldwide. There is a great need for the further development of tests for distinguishing reversal reactions from bacteriological relapses.

The Workshop emphasized the need for the careful investigation of all post-MDT relapse cases, according to standard protocols.

New Drugs

The Workshop welcomed the discovery and development over the last 5 years of the new anti-leprosy drugs. These include certain of the 4-fluoroquinolones, minocycline and clarithromycin. Studies on mice have shown these drugs to be both bactericidal and second only to rifampicin in their rates of killing *M. leprae*. Pilot clinical trials in lepromatous leprosy have been completed, and have confirmed that these new drugs are highly effective both clinically and microbiologically. The Workshop also noted the current work on other drugs, such as fusidic acid and the combination of brodimoprin plus dapsone.

There is now a need for the setting up of long-term clinical trials (in addition to the current ofloxacin trial) of a number of carefully selected regimens, noting both efficacy (judged chiefly by long-term relapse rates) and also drug interactions, toxicity, acceptability and effect (if any) on reactions.

These new drugs (ofloxacin, pefloxacin, sparfloxacin, minocycline and clarithromycin) should be used with care and caution, and not be given as monotherapy. In the short-term, they may prove important in the treatment of patients who are intolerant to 1 or more of the standard drugs, or who suffer from proven drug resistances (especially to rifampicin) or from intercurrent disease precluding the use of a standard drug.

The Workshop considered the possible value of chemoprophylaxis and immunoprophylaxis in areas of low and falling endemicity, but there were insufficient data on which to base recommendations.

Although the outlook for leprosy is now very hopeful because of the widespread application of MDT and the potential application of the new drugs, there is still a great need for continued long-term and careful chemotherapy work, both in leprosy control programmes and in the various integrated programmes.

Workshop 4 Reaction and Nerve Damage

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L. Lehman
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V. P. Shetty
J. N. A. Stanley
W. H. van Brakel

Progress in the Past 5 Years

CLINICAL

The use of graded, nylon monofilaments (Weinstein) has been accepted as the best way to monitor nerve function in an accurate and highly reproducible way under field conditions. With serial testing 'silent neuropathy' can be identified objectively and treatment instituted promptly. Serial testing can also identify therapeutic responses in silent neuropathy or overt neuritis.

Although much nerve injury occurs as overt neuritis during reactions, silent (asymptomatic) neuropathy is common and may occur either during a reaction or in the absence of any reaction.

In the treatment of reversal reactions, aggressive use of corticosteroid therapy has been found to be reasonably safe under field conditions, and need not be restricted to hospitalized patients. In erythema nodosum leprosum, if thalidomide is not available, corticosteroid treatment with clofazimine as adjunctive therapy is valuable. In either reaction for refractory patients the use of cyclosporin A or immunosuppressive antimetabolites has been shown to be effective.

With the use of operating microscopes, surgical decompression procedures are now associated with better results and reduced morbidity, and these operations should be reintroduced as a part of the established management of leprosy.

INVESTIGATIVE

Recent studies reaffirm the central role of delayed-type hypersensitivity to antigens of *M. leprae* in the immunopathogenesis of reversal reactions. Studies of T cell infiltrates in nerves and of the cytokines produced (in particular tumour necrosis factor alpha and IL-1 beta), are elucidating the mechanisms of nerve injury in reversal reactions.

M. leprae may also injure nerves by interference with Schwann cell metabolism, by eliciting antibodies, by stimulating autoantibodies, or by antigenic mimicry, via either antibody or T cell pathways.

Recommendations

DEFINITIONS AND TERMINOLOGY

For clarity and comparability a definition of 'silent neuropathy' is needed. A single standard name for reversal reactions would make this important problem accessible in Medline and other computer databases. A uniformly used terminology for disability, functional impairment, or nerve impairment is needed to establish comparability of clinical and investigative studies.

CLINICAL

For the early identification and prompt treatment of potentially reversible silent neuropathy or neuritis, standardization of nerve functional assessment and scoring is needed in the parameters of tests used, frequency of use and conditions of use. Once identified, recent onset neuritis or neuropathy should be promptly treated with an aggressive use of corticosteroids at an initial dose of 40–60 mg of prednisone or prednisolone daily with a slow taper after 4 weeks with monitoring of function (reduction of daily dose by 5 mg at 2-week intervals) until the level necessary to suppress the reaction is achieved. Treatment for 3–6 months or longer is needed. Caution should be exercised in patients with hepatitis B, strongyloides or tuberculosis. For instance, resting the limb is essential, with the elbow at not less than 110 degrees of extension.

If painful neuritis does not promptly respond to vigorous oral corticosteroid therapy or to parenteral dexamethasone administration, then surgical consultation regarding decompression should be sought before the injury is irreversible (ideally within 2–3 weeks of onset), but a favourable response to surgery may still occur after prolonged delay.

Following surgery or medical intervention, monitoring of nerve function should be carried out as long as the patient can be followed up.

FURTHER STUDIES

The identification of risk factors for neuritis would help its early identification and prompt treatment.

Because of its ability to inhibit tumour necrosis factor alpha, a known neurotoxin, a trial of thalidomide in non-ENL neuritis may be warranted.

Where possible, other methods of nerve assessment should be studied, such as laser-Doppler blood flow, electroneuromyography, or electronic vibrometry.

The use of corticosteroids in selected field areas should be monitored so that refined, better recommendations can be made for field use. Studies of other agents is encouraged.

Criteria or tests for the accurate differentiation between relapse and reactions (reversal or ENL) are needed for the increasing number of patients receiving short-term multidrug therapy (MDT).

Continued exploration of the mechanisms of reaction and nerve injury is needed. The devastating type II reactions in Latin America are a particularly vexing problem needing further study. Also, immunomodulating agents, such as drugs, mycobacterial components, cytokines or vaccines, should be developed for the treatment and prevention of nerve damage.

Since nerve injury or neuropathy may occur after completion of MDT, 3-month monitoring is necessary in paucibacillary patients for at least 2 years, and in multibacillary cases for at least 5 years.

Workshop 5 Experimental

Chair: Gerald P. Walsh
Rapporteur: Paul J. Converse

Participants:

Eduardo dela Cruz
Arvind M. Dhople
Robert H. Gelber
Bobby J. Gormus
Robert C. Hastings
Muhammad Ishaque
Tonetaro Ito
Charles K. Job
R. Denise McDermott-Lancaster
Richard W. Truman
Yasuko Yogi

Progress in the last 5 years

NUDE MICE

These athymic animals were first reported in leprosy studies in 1976 by Drs Kohsaka, Colston and colleagues. This model continues to be used in the evaluation of drug regimens for the treatment of leprosy because they can support the growth of large inocula to levels of 10^{10} per footpad. Dr McDermott-Lancaster (London) described how rifampin-resistant mutants selected in nude mice with a frequency of 1 to 8×10^7 viable organisms grow better in nude than in normal immunologically intact mice. Studies of anti-leprosy drugs combined with interferon-γ demonstrated a lack of synergy in reducing *M. leprae* growth except in combination with a therapeutic dose of rifampin but not with subtherapeutic doses or with DDS. A new anti-leprosy agent, ofloxacin, was found to be more effective when administered with daily dapsone than with monthly rifampin. It was found that *M. leprae* exposed to rifampin for 6 hours *in vitro* did not grow in nude mice whereas minimal killing by ofloxacin could only be detected after 96 hours exposure. As few as 10^1 to 10^2 organisms inoculated into nude mice, but not normal mice, showed growth over a 12-month period, demonstrating the value of nude mice in *M. leprae* viability studies. Professor Ito (Bangkok) reported that human-derived *M. leprae* multiplied as readily in nude as in normal mice. However, a strain of *M. leprae* (Thai-53) that had been passaged for many years in nude mice multiplied more readily in nude than in normal mice. Dr Hastings (Carville, LA) reported on a number of current uses for nude mouse-derived bacilli. As many as 10^9 *M. leprae* could be harvested weekly from nude mouse footpads for experimental use in metabolic studies, drug screening using radiometric methods, in culture with rodent Schwannoma cell lines, and in macrophage culture studies. Adoptive transfer studies using cells from Balb/c-nude heterozygotes immunized with a combination of *M. leprae* plus BCG resulted in the development of reversal reactions. In addition, transmission studies found that *M. leprae* applied and abraded (e.g., with thorns) onto cool but not warm skin resulted in growth of the organisms. This finding corresponds with observations made on armadillos caught in the wild that also showed evidence of *M. leprae* infection via contaminated thorns. The nasal mucosa appeared to be the primary site of infection in experimental transmission studies in nude mice. In chemotherapy studies, monthly rifampin by gavage was found to be less effective than daily rifampin in mouse food. Future studies in nude mice will evaluate new drug regimens for efficacy against persisting *M. leprae*.

BEIGE MICE

These immunologically-deficient (lack of NK cells, defective granulocyte chemotaxis, increased susceptibility to opportunistic infections) mice have been used for a number of years in biomedical research but are new in studies of leprosy. Dr Dhople (Melbourne, FL) reported that *M. leprae* multiplication in spleen and liver could be detected at least as early as 4 months in mice inoculated i.p. or i.v. Statistically significant enhancement of growth of *M. leprae* in footpads of beige compared to normal Balb/c mice was also observed. Dr Dhople also found the model to be suitable for chemotherapy studies.

SCID (SEVERE COMBINED IMMUNODEFICIENT) MICE

These mice have an enzymatic defect that results in a lack of functional T and B lymphocytes. They have attracted attention in recent years in infectious disease research due to their tolerance of functional xenogeneic mononuclear cells transplanted into the mice. Dr Converse (Baltimore, MD) summarized studies by investigators in Addis Ababa and Tokyo as well as his own that have shown that these mice are indeed susceptible to *M. leprae* infection when 5×10^3 to 10^7 bacilli are injected in the footpad. Dr Converse found that spread beyond the footpad to the popliteal lymph node and spleen could occur. In 1 mouse massive numbers of organisms were found in the nasal turbinates. Growth in SCID mice could not be enhanced by administration of a single dose of transforming growth factor b to abrogate NK cell function. Co-inoculation of *M. leprae* with cell-wall activated mononuclear cells from an *M. leprae* immune human donor but not a non-immune donor resulted in a reduction of organisms in footpad homogenates 3 months after infection. Dr Yogi (Tokyo) has observed dissemination of *M. leprae* after infection in the footpad. Dr Yogi reported that *M. leprae* inoculated i.v. resulted in significantly greater dissemination to footpads, bone marrow, liver, lips and ears of SCID mice than nude mice 14 months after infection. In addition, it was found by reverse transcriptase/polymerase chain reaction techniques that mRNA of cytokines that influence macrophage function was more detectable in 14-month infected SCID than nude hind feet whereas splenocyte cytokine mRNA was more readily detected in nude mice. In the experience of Dr Ishaque (Montreal) initial results also indicated greater susceptibility of SCID mice to *M. leprae* infection in terms of footpad swelling. However, subsequent experiments enumerating organisms found equivalent growth at 9 months and higher growth in the nude mice at 10 months after infection. Dr Ishaque was not able to detect bacilli in spleens or livers in SCID mice at this time. Dr Gelber (San Francisco, CA) reported that SCID mice reconstituted with immune T cells from Balb/c mice homed to the spleen, maintained long-term immune function, and limited multiplication of *M. leprae*.

NEONATALLY THYMECTOMIZED LEWIS RATS (NTLR)

Dr Gelber described earlier studies in which NTLR were proved to be a highly sensitive rodent model for detecting persisting *M. leprae* in patients undergoing initial chemotherapy. More recent studies using NTLR treated with various regimens involving newer anti-leprosy drugs demonstrated the value of this model in evaluating anti-persister drug regimens.

NORMAL MICE

Dr McDermott-Lancaster reported that sparfloxacin was superior to ofloxacin in infected mice at doses of 25 and 50 mg/kg by daily gavage for 60 days as determined by the proportional bactericidal test.

ARMADILLOS

This model was first described by Dr E. Storrs and Dr W. Kirchheimer in 1971. Dr Dhople discussed the history of the Florida armadillo colony, which has had contracts to supply infected tissues. More than 2300 armadillos have been inoculated for these projects. Until 1988 no problems with cultivable mycobacteria were encountered. Since then cultivable organisms have been found in approximately 30% of animals with disseminated leprosy. There has also been a decrease in the yields of *M. leprae*. The workshop participants discussed possible causes of and remedies for these problems in addition to the intensive efforts already undertaken by Dr Dhople and colleagues. Dr Dhople also confirmed previous observations of Dr J. Convit that Venezuelan 9-banded armadillos are not as susceptible to *M. leprae* infection as armadillos from the USA. None of 25 Venezuelan armadillos developed disease while 70% of USA armadillos inoculated with the same suspension developed leprosy. Dr Dhople found that armadillos metabolize dapsone in a manner very similar to humans. Biopsies of cutaneous samples obtained from infected armadillos receiving DDS revealed decreasing ATP levels during the course of treatment.

Dr Truman (Carville, LA) presented a comprehensive report on the history, distribution, migration, physiology and husbandry of armadillos. The epidemiology of sylvatic leprosy in armadillos in the USA appears to follow a corridor along the southern Mississippi River valley and then along the Gulf coast to the Mexican border. The prevalence of infection in adult armadillos is estimated to be 30% in this corridor. This pattern correlates with the distribution of indigenous human leprosy in the USA. He also reviewed the pathogenesis of *M. leprae* infection in experimentally-infected armadillos. As few as 10^3 organisms can result in a successful infection but typically 10^8 are inoculated i.v. in order to shorten the incubation period to an average of 14 months. Early indications of 'takes' are a nodule at the injection site, IgM antibodies to PGL-1, and detection of *M. leprae* specific DNA in PCR. Usually there are few clinical signs of infection. If cutaneous lesions develop and ulcerate, they represent a source of organisms in the environment. Dr Job (formerly Carville, LA) reported that armadillos whose reactions to lepromin had a histological resemblance to tuberculoid leprosy were usually resistant to subsequent infection whereas those with a lepromatous type response were the most susceptible and 6 weeks after immunization with either 10^7 BCG or 10^7 BCG plus $1{\cdot}6 \times 10^7$ heat-killed *M. leprae*, lepromin conversions were observed in 20% of armadillos. Only 3% of control armadillos converted. Dr Job also found that footpad infections were more successful with larger inocula and that infections proceeded from footpad granulomas and then to regional lymph nodes, the spleen and finally other organs. Dr Job pointed out that at a pre-clinical stage *M. leprae* is found in the reticuloendothelial system before invading the nerves. Finally, 2% of 'road-kill' armadillos were found to have disseminated disease, suggesting the potential for trillions of organisms to be discharged into the environment allowing spread to other hosts by means of skin abrasions.

CYNOMOLGUS MONKEYS

Dr dela Cruz (Cebu, the Philippines) described experimental studies in Philippine cynomolgus monkeys. Thus far, 4/22 animals have developed AFB positive nasal smears after inoculation with *M. leprae* and the positivity of the nasal mucosa correlates well with PGL-1 antibody levels in these animals. PCR examinations of nasal smears were positive in the 3 animals with available specimens as well as in 2 additional inoculated animals that were AFB negative by conventional methods. Sooty mangabey monkey isolates containing Simian Immunodeficiency Virus (SIV) were used to infect several groups of cynomolgus and the presence of SIV appears to enhance susceptibility of this species to leprosy. Surveys of ferral cynomolgus monkeys revealed serological evidence of natural leprosy in 3/596 monkeys. Additional studies of these 3 animals as well as more ferral monkeys are in progress.

CHIMPANZEES

Dr Gormus (Covington, LA) reviewed naturally acquired leprosy in 3 chimpanzees. The first chimpanzee with leprosy was from Sierra Leone and had been in the USA only a short time before diagnosis. The remaining 2 chimpanzees had been in the USA for more than 10 years before diagnosis and retrospective serological studies revealed that the disease was probably incubating in these animals when they were imported from Africa. Current efforts involve serological testing of chimpanzees maintained in various colonies for other biomedical investigations.

OTHER OLD WORLD MONKEYS

Naturally-acquired leprosy was described in 2 sooty mangabey monkeys (SMM). It is likely the first mangabey acquired the disease in Africa and was the source of infection for the second animal with whom it was caged for a number of months. This appears to be the first case of monkey to monkey transmission. Experimental leprosy studies in SMM revealed that this species was very susceptible to leprosy. Although dose–response studies demonstrated a variety of individual responses in terms of time and extent of disease, the SMM is undoubtedly the most susceptible non-human primate species studied to date. Variations in the course of leprosy in inoculated animals may be reflected in the cyclic variations in lymphocyte responses to mitogens observed in normal and *M. leprae* infected SMM. Experimental studies also demonstrated that rhesus monkeys were susceptible and developed BB to LL leprosy after infection with *M. leprae*. Captive SMMs are asymptomatic carriers of SIV but rhesus monkeys inoculated with SMM isolates develop simian AIDS together with leprosy. Many succumb to SAIDS-related opportunistic infections. SIV appears to enhance susceptibility of rhesus monkeys to leprosy. Experimental studies in African green monkeys demonstrate that they will develop a BB/BL form of leprosy involving primarily nerves.

Future Directions

Recognizing the contribution of animal models and their continuing role in providing information on the epidemiologic, chemotherapeutic, immunologic, microbiologic, and pathogenic aspects of leprosy, the following aims for future work are recommended.

1 Additional studies in SCID mice should be carried out to resolve questions concerning *M. leprae* dissemination, reproducibility, and the overall utility of the model including vaccine and cell transfer experiments.
2 Nude mice continue to have value (a) as a source of viable *M. leprae* for *in vitro* and other experimental studies (e.g., transmission and animal inoculations), (b) as a relatively inexpensive model for chemotherapy studies, and (c) as a means of detecting small numbers of viable bacilli.
3 Future studies in beige mice will involve histopathology and additional experiments on the pathogenesis of leprosy infection.
4 The normal mouse footpad assay remains the most readily available model for viability, drug screening, and drug sensitivity testing.
5 The NTLR will continue to be used in *M. leprae* persister studies.
6 The armadillo has as yet untapped potential in studies of the transmission, epidemiology, chemotherapy, and immunology of leprosy. Investigations of these various aspects of leprosy in wild-caught animals should be continued. This model's potential for assessing candidate vaccines and new drugs may be particularly worthwhile areas to investigate. New techniques in assisted reproduction may overcome the present barriers to breeding in captivity. Understanding the differences in susceptibility of Venezuelan and North American 9-banded armadillos may be a rewarding avenue of investigation.
7 In non-human primates, studies should: (a) ascertain natural infection in ferral and captive primates and investigate the contribution of retroviruses to the development of leprosy in these species; (b) continue studies evaluating the susceptibility of cynomolgus monkeys to experimental infection with *M. leprae*; (c) continue to evaluate the relevance of the SSM vaccine model; (d) continue to study the African green monkey as a model for neuritic leprosy; (e) investigate *M. leprae* strain differences in the pathogenicity of infection in rhesus monkeys as a model to determine if strain differences play a role in the spectrum of human leprosy.

Workshop 6 Pathology

Chair: Ashok Mukherjee
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Participants:

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Charles Pangi
David Scollard
Vanaja P. Shetty
F. Takahashi

Summary and recommendations

Pathology continues to make an important contribution to the study and control of leprosy. Although the histopathology of leprosy is well known, there are a number of

areas in which recent advances have been made and some in which the pathologist should respond to advances in other disciplines. In leprosy pathology, as in many other fields of leprosy research, there is a need to distinguish cause from consequence. The workshop makes the following recommendations:

We recommend the application of standard diagnostic criteria for early leprosy.
2 Planning and implementation of all new trials of treatment should include a pathologist.
3 For the distinction between relapse and reaction, demonstration of solid-staining AFB appears to be the most reliable histological criterion.
4 There is an urgent need for detailed pathogenetic studies of the Lucio phenomenon.
5 In view of the global pandemic of HIV infection, the relationship between AIDS and leprosy requires careful clinico-pathological study.

Early leprosy

The diagnosis of early leprosy is often uncertain clinically and histologically. However, there are many patients in which histology either confirms the suspicion of leprosy or provides an alternative diagnosis. Histological examination of clinically indeterminate lesions and lesions suspected of leprosy is an important diagnostic tool. However, individual pathologists differ widely in the certainty with which they diagnose leprosy and there is clearly a need for the standardization of diagnostic criteria. The following criteria should be applied for the diagnosis of biopsies from clinically suspicious lesions of leprosy:

(a) A diagnosis of early leprosy can be given if one or both of the following criteria are satisfied.
(i) The presence of convincing acid-fast bacilli (AFB): the participants agreed that a minimum of 6 serial sections of every biopsy should be stained by an appropriate modified Ziehl–Neelsen method such as the Wade–Fite or Fite–Faraco for AFB. Certain sites are more likely to harbour AFB and should be searched in the following order: nerve-bundles, sub-epidermal zone, arrector pili muscle, and areas of inflammation.
(ii) The presence of endoneurial inflammatory cells (lymphocytes and macrophages usually predominant), preferably with disruption of neural architecture.
(b) A report of findings compatible with leprosy should be given if the following suggestive features are present.
(i) A chronic mononuclear cell inflammatory infiltrate with a superficial and deep dermal pattern surrounding nerves, vessels and adnexae, without neural disruption or AFB. In such cases, re-examination for AFB and possibly further biopsy is indicated.

Procedures which may be of help include the use of sections cut at deeper levels to determine the relationship of inflammatory foci to nerve. Special staining procedures (e.g. S100 and Neuron-specific enolase for nerves) and the demonstration of mycobacterial antigens may be helpful with appropriate controls, but require further evaluation.

Biopsies should generally be taken from the edge of the lesion, but in lesions less than 1·5 cm diameter, a central biopsy is more likely to be diagnostic. The diagnosis and classification of established leprosy is not usually problematic. In evaluating the

differential diagnosis of granulomatous dermatitis the above principles apply. Clinical consultation is an important part of the diagnostic process.

Evolution of Disease

Several presentations addressed the issue of disease progression in skin, nose and nerve. There is scope for further research in this area and for sequential biopsy studies. Bacilliferous lesions may be present in the nasal mucosa and nerve prior to the development of skin lesions. It is possible that early nerve damage occurs in the absence of bacilli, but further work on the pathogenesis of nerve destruction before, during and after treatment is required, ethical considerations permitting. In particular, the pathogenesis of progressive neural deficit and fibrosis following cessation of therapy requires elucidation. The interaction between *M. leprae* and endothelial cells may be an important determinant of localization and lesion development. Further work is also required in this area.

Reactions

The basic criterion for diagnosis of erythema nodosum leprosum (ENL)—infiltration by neutrophils—is well known, but in a proportion of cases with clinical evidence of ENL, no neutrophils are present. Participants felt that this discordance might be due to timing of the biopsy and the importance of changes in vascular dynamics which are not seen by the pathologist. ENL has an appreciable mortality rate and in these severe cases, ENL lesions are often found in internal organs at necropsy. The pathogenesis of ENL requires clarification in relation to neuritis, glomerulonephritis, iridocyclitis, arthritis, testicular involvement, amyloidosis, immune complexes and reversal reaction.

The clinical and histological features of the Lucio phenomenon have been fully described, but the pathogenesis of this reactional state remains obscure. Clearly, vascular bacillation and associated thrombotic phenomena are important. However, the genetic and immunological factors involved need substantial research investment.

The histological diagnosis of reversal reaction is often difficult and does not appear to correlate well with clinical signs. This may reflect the lack of histological features associated with erythema and induration. However, immunological changes such as increased IL2R or HLA-DR expression and CD4+ lymphocyte infiltration can be seen and quantified in histological sections. Reversal reaction may represent qualitative or quantitative immunological differences among patients. An effort should be made to distinguish between both possibilities. The relative importance of other factors such as disease load, treatment, timing of biopsy, sex and age has yet to be determined.

The participants were unanimous in their opinion that relapse could only be reliably distinguished from reversal reaction following MDT when solid-staining AFB are demonstrated. The most difficult biopsies are those in which there are no bacilli and the appearances are of tuberculoid type.

Monitoring treatment

In clinical trials, the response to therapy should be monitored by histological as well as clinical, bacteriological, and immunological means. There was concern at the lack of

histological evaluation in several current chemotherapeutic trials. The inclusion of a pathologist at the planning stage of these trials is a necessity.

Recent studies have demonstrated the utility of sequential biopsies to measure many parameters, including granuloma fraction, bacterial index, antigen load, and cell surface antigen expression. These can be used as surrogate markers of response to chemotherapeutic and immunochemotherapeutic regimens.

After completion of MDT, there is often persistence of foam cells without solid bacilli (MBL) or epithelioid granulomas (PBL) in the absence of clinical activity. The significance of these changes is not understood, and there is no consensus as to whether histological normality should be a condition for release of patients from treatment.

Systemic involvement

Systemic involvement in leprosy is common, particularly in lepromatous patients. Regression in the skin may occur during treatment without complete clearance of visceral, ocular and neural bacilli, leading to later relapse. Involvement of the larynx and testis are of particular importance. Secondary amyloidosis occurs which can best be diagnosed by the biopsy of minor salivary glands, and may regress following treatment.

Eye involvement is common, but usually seen by the pathologist at a late stage in its development. There is a need to follow ocular changes by regular examination during treatment and to obtain material for pathology where possible.

The pandemic of HIV infection and AIDS involves many leprosy endemic areas. The effects of HIV on leprosy are not yet clear and require further clinico-pathological study.

There is need for all tissue removed from leprosy patients to be sent for pathological examination and for further necropsy studies to be performed.

Training, quality assurance and audit

The workshop identified a lack of trained leprosy pathologists, particularly in endemic countries. There is an urgent need to educate practising and trainee pathologists in the diagnosis and classification of leprosy. One method is the organization of regular training workshops. In many countries technical standards of skin section preparation require improvement. This could best be achieved by visiting senior technical staff from existing laboratories. Achievement of these aims could be assisted by the development of quality assurance and audit schemes for histopathology laboratories.

Workshop 7 Training of Professionals

Chair: Djohan Kurnia
Rapporteurs: Carmen Bueno
Heather Currie
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Participants:

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S. J. Nkinda
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Introduction

The Workshop on Training (as well as on other topics in leprosy control) has been a standard feature at the International Leprosy Congress for many years.

The Workshop on Training of Professionals in Leprosy before the IVth ILA Congress was attended by trainers from all parts of the world and 20 papers were presented.

With reference to the global MDT situations, the workshop reviewed the current status of training in various areas and made some recommendations to ensure that training activities were more effective and efficient in assisting the leprosy control programmes toward the goal of eliminating leprosy by the year 2000.

OBJECTIVES

The objectives of the workshop were:

(1) to review the present status of the training activities for the professionals in leprosy in the world;
(2) to identify important unresolved issues in the training of professionals in leprosy;
(3) to recommend guidelines for approaches to resolve these issues.

REVIEW OF THE PROGRESS

During the last 5 years, progress has been made in the field of training in the following areas:

1 *Approach in learning and evaluation methods*
Learning in leprosy is moving mainly from subject to task orientation using problem-based, student-centred methods.

2 *Emphasis on curricula priorities*
In addition to the basics of clinical leprosy and control many training programmes are

realizing the need to include better management training, more emphasis on communication skills, training in prevention of disabilities and health system research.

3 *Training of trainers*
In recognition of the need to upgrade the education skills of trainers, some workshops were organized at national and international levels.

4 *Production of materials*
The use of modular training material has increased. Local production and adaptation has been encouraged. In some countries, materials have been produced for undergraduate healthcare workers.

Recommendations

Although progress has been made in the areas mentioned above, the participants feel it is essential to continue to strengthen and consolidate what has already been achieved.

The following recommendations are made for the future of the training of professionals in leprosy:

Approach in learning and evaluation methods

1.1 Strengthening of the problem-based, task-oriented approach to learning needs to be continued. Education should be continuous. Learning can continue in the field using self-learning materials, distance learning, training follow-up and feedback.

1.2 There should be a multidisciplinary team approach to training.

2 *Emphasis on curricular priorities*

2.1 Training needs to be tailored to programme needs, e.g., in areas where MDT coverage is low, emphasis could be placed on the implementation of MDT, early detection and complete treatment. In some areas, courses held in special training centres can be shortened. In areas of high MDT coverage, training could concentrate on prevention of disabilities, vocational training and all aspects of rehabilitation. The hidden programme needs, which are often neglected, should be emphasized, such as patient education, communication skills, management and psycho-social aspects. As we move toward the elimination of leprosy, it will be necessary to develop appropriate training strategies to cope with integration, e.g., training primary healthcare workers, medical students, combined TB-leprosy courses and community-based rehabilitation. This will involve the production of materials and training modules for undergraduates in medical fields.

3 *Training of trainers*

3.1 Training capacity can be multiplied through training of trainers. Specialized centres should equip regional training teams to pass on their knowledge and skills.

3.2 Training centres should be linked so that there is a networking of expertise and

sharing of developments and ideas. This may entail the identification of global co-ordinating centres.

4 *Production of materials*

4.1 Appropriate training materials need to be produced. The distribution of these materials needs to be rationalized.

5 *Selection of students*

5.1 This is an area that needs to be looked into. We would recommend that criteria for selection be drawn-up by centres. Selection of trainees should be based on local needs.

All these recommendations depend on an increase in resources, which will necessitate a channelling of funds into training and material production.

Workshop 8 Approaches to Epidemiology, Prevention and Control

Chair: Kumar Jesudasan
Rapporteur: M. D. Gupte

Participants:

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A. Meima
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Vijayakumaran
H. Wiher

There has been a significant fall in the prevalence of leprosy in all parts of the world where MDT has been implemented. There has been a decrease of nearly 60% in registered cases between 1985 and 1993. The estimated number of cases has also fallen from 11 million in 1985 to 3·1 million in 1993. The statistics for 1993 on registered cases indicate that 80% of the registered cases of leprosy come from India, Brazil, Nigeria, China and the Sudan. In 1991, the World Health Assembly adopted a resolution on the elimination of leprosy as a public health problem. Elimination was defined as a prevalence of less that 1 per 10,000.

Our current understanding of the epidemiology of leprosy has not changed significantly since the last congress. No new light has been shed on factors relating to the transmission, evolution or any other factors relating to the epidemiology of the disease.

There is a need to have simplified indicators relevant to leprosy control and which are based on minimum and essential data. Measurements of trends of leprosy should ideally be done using incidence rates. The true incidence and prevalence of leprosy are difficult to

measure and case detection rates and prevalence rates should be used for following trends in leprosy, taking care to use correction factors by adopting the appropriate adjustments.

Findings from Venezuela on the immunoprophylactics with BCG and killed *M. leprae* (armadillo derived) combination have been inconclusive. Results from other vaccine trials using BCG and killed *M. leprae* and other combinations such as ICRC, *Mycobacterium w*, are expected to be available after 1995. As of now no second generation vaccines against leprosy are available. The present priority for immunoprophylaxis should be to complete the current vaccine trials and to assess their outcome before considering new trials.

While chemoprophylaxis may have a limited role in individuals, it is not a tool that can be recommended for the prevention of leprosy at the community level, therefore the mainstay for leprosy control is chemotherapy. Thus the priority for leprosy control is to increase MDT coverage in all parts of the world and ensure a high case detection level.

While looking into the effect of MDT on the trends of leprosy, it can be seen that in many areas the case detection rates (proxy for incidence rates) have stabilized and are not showing signs of decline, even after the implementation of MDT for between 8 and 10 years. For instance, in some hyperendemic areas in India the stabilization has been in the region of 1 per 1000. There is a need for a health systems approach to investigate this phenomenon in the light of the proposed target of the elimination of leprosy by the year 2000.

Better strategies for leprosy control can be discovered by improving our knowledge of the epidemiology of the disease. A test to identify *M. leprae* infection is expected to help in achieving this goal and should be given high priority in research. The tools based on molecular biology and immunology, as well as newer tools, should be investigated further in this respect. Epidemiological studies need to be taken up to establish the magnitude and dynamics of infection. A holistic approach to understand the micro-epidemiology of leprosy is needed and such work can be done in sentinel centres with appropriate samples of population. The interruption of leprosy transmission in the community will depend on the identification of all leprosy cases and their effective treatment. The proportion of leprosy cases remaining undetected (unknown patient load) would contribute to continued transmission. It is also necessary to look into the possibilities of non-human reservoirs for *M. leprae* in this connection. This would involve the collection of epidemiological, immunological, bacteriological, environmental, socio-economic and behavioural data from selected areas of high and low endemicity from different parts of the world. Establishment of these sentinel centres are of paramount importance.

There is a need to develop appropriate epidemiological indicators to study leprosy epidemiology after the level of elimination is reached.

Prevention of disability starts with the control of leprosy. Nevertheless the occurrence of disability in patients on treatment and new disability after release from treatment is a cause for concern. The Sixth Expert Committee on Leprosy (WHO) has recommended that prevention and management of impairments and disabilities, which have long been recognized as essential components of leprosy control programmes, should be implemented effectively. Leprosy is a public health problem because of the deformities it causes and there is a need to give very high priority to disability prevention. The possible use of disability rate and occurrence of new disability during treatment and after cessation of chemotherapy should be considered for evaluating the quality of leprosy control.

The development of epidemiological models is important in order to predict and

simulate the trends of leprosy under various operational conditions. This exercise should be related closely to the actual implementation and monitoring of leprosy control activities.

There has not as yet been demonstrated any relationship between HIV infection and the risk of leprosy or susceptibility to any particular type of leprosy. The effect of HIV infection on relapses, reactions and neuritis needs to be studied.

The area of urban leprosy control needs to receive special emphasis in view of the rapid growth of cities and migrations from the countryside to urban slums. Efforts should be made to trace such migrant cases as soon as possible and treat them with effective MDT. Health systems research could contribute to our understanding of the dynamics of urban leprosy and thus facilitate the evolution of new strategies for urban leprosy control.

This workshop ended with the optimistic view that a concerted approach will help us to develop a better understanding of the disease, which in turn will help us formulate improved strategies for the elimination and eventual eradication of leprosy.

Workshop 9 Consumer and Community Participation in Care and Rehabilitation Programmes for Persons with Leprosy

Chair: Maria Leide W. de Oliveira (Brazil)
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Rapporteur: Judith Justice (USA)

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Anthony Samy (India)
Ram Kumar Shrestha (Nepal)
Jagdish Sircar (India)
Heather Smith (Thailand)
Samuel Solomon (India)
H. Srinivasan (India)
Alec Style (U.S.A.)
Elsa Taferi (Ethiopia)
Li-He Yang (China)
Jin-Sang Yoo (Korea)

Introduction

The Workshop on 'Consumer and Community Participation in Care and Rehabilitation Programmes for Persons with Leprosy' combined 3 Workshop topics from the XIIIth International Leprosy Congress, including Social Aspects, Health Education and Rehabilitation.

The emphasis of the Workshop was on the individual who is suffering from the disease and the community as consumers and the Leprosy Control Programme as the production process which encompasses all aspects of care, extending beyond chemotherapy and the reduction of prevalence rates. The Workshop emphasized the social and economic aspects of leprosy without ignoring the bio-medical aspects. In keeping with this approach, Social Science Research needs to provide the appropriate theories and methodology for studying the Leprosy Control Programme with special efforts to include perceptions of individuals with the disease and the community.

The following invited formal presentations were made by Workshop participants:

'Status of Health Education in Leprosy Control' by Dr R. Mutatkar (India);
'Physical Rehabilitation and the Community' by Dr H. Srinivasan (India);
'Socio-Economic Rehabilitation' by Dr J. Lew (Korea) and Mr S. K. Jung (Korea);
'The Priorities of Social Science Research in Leprosy' by Dr Judith Justice (USA);
'Case Study of Economic Rehabilitation in India' by Dr Jal Mehta (India);
'Hawaiian Storytelling' by Makia Malo (USA).

Discussion

The Workshop participants were divided into 3 discussion groups: Health Education, Rehabilitation and Social Science Research.

The Health Education Group addressed the following questions.

How can leprosy treatment (in its present short-term form) be managed so that the individual's life, work and family relationships will not be interrupted? Has MDT really altered the social isolation felt by individuals suffering from leprosy? What can be done to lessen their social isolation?

How can individuals with leprosy be brought into the public health education process?

The Rehabilitation Group addressed the following questions:

Can Leprosy Rehabilitation Programmes be successfully integrated into the General Health Care System? If so, how would this be possible? Is this always desirable?

How can individuals who have suffered from leprosy, and have been living in colonies or other isolated situations, be empowered to participate in community activities? How can they be re-integrated into the community and what can be done for those for whom integration is not possible?

How can individuals with leprosy improve their socio-economic status in order to become fully accepted as productive members of the community?

How can fund-raisers be brought into the public education process?

The Social Science Research Group addressed the following questions:

What should be the priorities of social science research in leprosy?

How can the results of the projects in social science research be applied in order to improve the efficiency of leprosy control?

Recommendations

RECOMMENDATIONS FOR HEALTH EDUCATION

1 The management of leprosy treatment can be successful at the individual and family level without interrupting normal life, work and family relationships. To achieve this, drug delivery and other services should be combined with personal and family counselling and appropriate health education for the individual, his family and the community.

2 A combination of MDT and health education has reduced the social isolation felt by individuals with leprosy.

3 Health education efforts should be periodically evaluated and updated to include the latest scientific information.

4 As leprosy programmes are increasingly integrated into the general health care system, the number of leprosy workers and health educators is often reduced. Serious consideration should be given to training individuals suffering from leprosy to fill these positions so that health education efforts are not diminished.

5 Networks should be devised whereby information can be distributed to individuals with the disease.

6 It is recognized that each country has its preferred terminology for the disease. However, the use of derogatory terms such as 'leper' should *never* be used.

7 In integrated programmes, community participation becomes even more necessary. Organized community groups such as youth groups, women's groups and service organizations, should be utilized to help disseminate information on leprosy. Information provided should take into consideration socio-cultural factors of the people.

8 Religious leaders should also be encouraged to be health educators to educate the community about leprosy.

9 Every effort should be made to ensure that the mass media does not give incorrect information which perpetuates stigma and fear.

10 Ultimately, the responsibility for health education should be transferred from health providers to the community.

11 Health education will help to achieve the stated goal of the elimination of leprosy, but this must be continued even after leprosy is no longer considered 'a public health problem'.

Recommendations for Rehabilitation

Multi-Drug Therapy has brought great benefits to a large number of people and future development in chemotherapy promises simpler and shorter treatment. These changes suggest a possible shift in emphasis and increased allocation of human and financial resources to the physical and socio-economical rehabilitation of individuals who have leprosy-related handicaps. The participation of individuals with such handicaps in the delivery of rehabilitation services at all levels is seen not only as a response to their demands, but also as a beneficial contribution. In view of such future developments, the participants of the discussion group on Rehabilitation made the following recommendations:

Each country should develop a national policy suited to its requirement to deal with leprosy-related problems other than chemotherapy. Such a policy should make a political commitment to deal with the massive problems of physical and socio-economic rehabilitation, allocate necessary funds, generate the needed infrastructure and ensure the participation of people with leprosy-related handicaps in the efforts of governments and non-governmental organizations.

2 Action plans for physical, socio-economic and psychological rehabilitation of the individuals with leprosy-related handicaps need to be prepared and adequate budgetary allocation ensured.

3 It is strongly recommended that, wherever possible, physical rehabilitation and disability prevention programmes operate through the general health delivery services. With MDT, contact between providers and persons with the disease is reduced, increasing the risk of neglect of disability prevention. To achieve effective implementation of programmes, appropriate training should be given to all connected personnel.

4 It is recognized that leprosy and poverty are inter-related. Therefore, stigma associated with the disease can more easily be eliminated through improving the individual's economic status rather than through education of the community. Experiences in Korea and India confirm that large-scale initiation of programmes designed to improve the socio-economic status of individuals with leprosy-related handicaps can be very successful and are needed urgently in other areas.

5 In countries such as Japan and the USA where economic well-being is not as serious a problem as in developing countries, individuals with leprosy still encounter problems of self-respect related to society's attitudes. For example, Japanese laws require individuals with leprosy to inform officials every time they travel, even within the country. The Workshop therefore recommends that discriminatory laws such as in Japan and India be removed and individuals with leprosy-related handicaps be respected.

6 Socio-economic projects have a greater chance of success if they are planned and controlled by the beneficiaries. Modes of control, format and management may vary, e.g. handicraft subcontractors, agro-based groups, co-operatives, registered small-scale industry, etc., depending on the situation in each country, with the common factor being full participation of the beneficiaries in the decision-making process.

7 'Leprosy colonies' need not always be viewed as a negative development but one that fulfilled a need and therefore the situation must be utilized to the best advantage. An extreme example is Korea where the non-disabled moved into such villages because of their economic prosperity. Brazil provides another example where people in colonies are free to leave, and those who choose to remain must be allowed to live undisturbed. However, it must be strongly stated that *no new colonies or segregated hospitals or settlements* should be created.

8 In countries where special privileges are given to persons with hearing, visual and orthopaedic disabilities, similar privileges should not be denied to those with leprosy-related social disabilities.

9 The use of photographs and pictures depicting poverty, deformity and deprivation that shock people, promote pity and appeal to emotions should not be used in fund raising. The information provided today should depict reality and appeal to the intellect. In practical terms, earlier appeals were for grants for charitable purposes. Appeals should now be based on the need for investment in development and how this involvement would provide relief in the short-term and eliminate dependency in the long-term.

Recommendations for Social Science Research

Recommendations were made taking into account the individual, community, health services, technology, and social science theory and methodology.

1 The group endorses the definition of leprosy as given in the Sixth WHO Expert Committee Report on Leprosy. Social science research, therefore, should address all aspects of leprosy, including epidemiology, chemotherapy, deformity prevention and rehabilitation.

2 Social science research should document and evaluate the process of integrating leprosy control programmes into the general health services.

3 Social science research should address the concept of cure, including the perceptions of patients, community and health workers, particularly related to the implementation of MDT at the global level.

4 Social science research methodology workshops should be held for leprosy workers who are interested in social science inputs in leprosy control work.

5 Training materials should be developed for medical officers and other health workers to look at problems of individuals with the disease and of the community, which result from disability, ulcers, threat of social isolation and dehabilitation.

6 Social science research should be used to help individuals with leprosy.

7 A recording system for non-medical work needs to be developed, including qualitative and quantitative parameters.

8 Funding for social science research in leprosy needs to be generated from international and national agencies, including the international organizations such as WHO, UNICEF, ILA, ILEP, ALM, national and international NGOs, and national social and medical research councils.

9 Social science research should be used for research, documentation and evaluation of community participation programmes designed to share responsibilities of the health services in leprosy control.

10 Social scientists should study social, economic and political issues related to the decline of leprosy colonies.

11 Participatory research approaches involving individuals with leprosy, community and health workers should be encouraged.

12 Social scientists should give special attention to identifying research problems and implementation of studies in collaboration with individuals, the community and health care providers.

13 Research results should be disseminated in accessible forms, including publication in international and national scientific journals, particularly in countries where research is conducted, and made available to patients, communities and leprosy control programmes.

14 Because it is not possible for many social scientists to attend international meetings, national and international networks of social scientists should be formed to enhance the training of social scientists, to increase social science research, and to ensure the dissemination of research results. Social scientists should be recognized as one of the scientific groups of the International Leprosy Association with specialized meetings at the International Congress.

Workshop 10 Management of Physical Disability

Chair: Frank Duerksen
Rapporteurs: Dinkar Palande
Roland Kazen

Participants:

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Atul Shah
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Marcos Virmon
Jean Watson
Ruth Winslow

Although multidrug therapy (MDT) has changed the bacteriological aspect of leprosy, the position regarding deformity and disability remains the same. For a patient with a deformity and disability the cure consists of either its prevention or its correction.

In the last 5 years there has been an increasing recognition of the importance of including prevention of disability (POD) as an essential part of leprosy management.

Within all leprosy rehabilitation theories are world views which must be addressed within the social, cultural and psycho-social context.

The workshop considered the following issues:

(1) prevention of disability (POD);
(2) early detection of nerve damage;
(3) monitoring;
(4) training;
(5) care after cure.

1 Prevention of Disability

The prevalence of patients with impairment continues to rise over the years. The incidence of disability has only been slightly diminished, therefore the number of patients in need of disability control is still very high.

The most common use of the WHO disability grading is as a new case-finding indicator. If it is to be used to determine disability caseload, it is essential that discharged patients and grade 1 disabilities are included. WHO grading is neither intended nor appropriate for monitoring changes in levels of impairment. For this purpose more detailed measurable clinical information is needed.

Leprosy control programmes need to secure funding and ensure effective training of staff in the early identification and monitoring of impairment and prevention of further deterioration.

One of the tried methodologies is given in the ILEP publication 'Prevention of Disability, Guidelines for Leprosy Control Programmes' (March 1993).

It has to be recognized by all concerned that impairment of function can occur before, during and after chemotherapy.

Public awareness has an impact on early detection and consequently on prevention of

disability. Disability prevention and rehabilitation ensures the credibility of leprosy control programmes. The number of presentations under rehabilitation in this Congress attests to the increasing awareness of its importance.

2 Early detection of nerve damage

Early detection of nerve damage is the key to prevention of deformity and consequently of stigma in leprosy. Adequate training of the staff in the evaluation of clinical, sensory and motor aspects of nerve function is essential and feasibile.

Motor and sensory testing by quantitative methods is available and feasible under field conditions, and essential for proper monitoring of nerve damage. Tests should be performed at the time of diagnosis and periodically during and after treatment. We recommend the use of the well-documented and widely used standardized graded nylon monofilaments for sensory testing.

Methodology should be adapted to regional conditions, but it is essential that some type of periodic quantitative assessment is used.

A patient with any clearly defined loss of sensation in the eyes, feet and hands has to be regarded as being at high risk to suffer further deterioration and consequently will need careful monitoring.

The patient's awareness of the risk factors and self-examination is essential to ensure voluntary reporting.

3 Monitoring

It is time that the leprosy programme manager assumes responsibility for disability control during and after treatment. This includes monitoring of:

1 vision, wounds and cracks of any insensitive parts;
2 needs of footwear, its acceptability and distribution;
3 ongoing self-care programmes; and
4 the required protective and adaptive appliances.

4 Training

The need for training includes the patient, his family, the community and staff.

THE PATIENT

The patient should be trained to recognize symptoms and signs of early nerve involvement and the deterioration of any present condition, especially of his eyes, feet and hands. The patient also needs to be trained in self-care and in the use of protective measures. The training is not complete until the patient's compliance is observed.

THE COMMUNITY

The community needs to be made aware that persisting deformities in a cured patient do not indicate any threat to the public.

STAFF

Staff should be trained to recognize impairment, disability and handicap, respond to the patient's needs and work with his/her initiative to overcome them.

5 Care after cure

Irrespective of chemotherapy, patients will continue to develop impairments and are in need of care almost indefinitely. It is suggested that patients with risk of, or with any grade of disability, are entered into a separate register after release from treatment (RFT). This is to ensure that there is a mechanism in place for further follow up, if necessary. It will also ensure that the patients do not feel unwelcome when they report any complications that arise.

Rehabilitation services should progressively be integrated into general community services.

Leprosy rehabilitation personnel should strive towards 'reverse integration' and teach colleagues in general health services the basic techniques known to work in leprosy.

Workshop 11 Guidelines for Leprosy Control Managers

Chair: P. Feenstra
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Introduction

The current MDT regimens for MB and PB leprosy are appropriate for routine field conditions. Experience has shown that they are effective, safe and operationally feasible. They are acceptable to patients and field staff.

Regular and complete treatment with MDT of all known leprosy cases and early diagnosed new cases is the most cost-effective element of the strategy to achieve the leprosy control objectives. Therefore the establishment of early case finding and treatment with MDT remains the top priority for leprosy control programmes. The diagnosis can be made with simple techniques in the vast majority of cases and there are

only 2 groups within the classification, each with its own standardized treatment which can be safely applied under field conditions.

In April 1993 the total number of leprosy cases was estimated at 3·1 million in 90 endemic countries of whom 2·3 million were registered for chemotherapy. The MDT coverage varies widely between the leprosy endemic countries and within individual countries. The fall in MDT coverage from 55% in 1990 to 48% in 1993 indicates that it has become more difficult to reach the remaining patients. The reasons for this failure to consolidate MDT implementation are multiple and vary from country to country and also within individual countries. The major explanations are: lack of political commitment, competition with other health problems in the countries, weak management capacities and organizational problems of the health services, inadequate training in leprosy of general health staff, lack of resources, lack of an appropriate plan of action and/or an operational manual, poor referral facilities and the rigid and demanding requirements for the introduction of MDT which were identified in the early 1980s.

Far too many leprosy patients do not yet have access to the benefits of MDT. Successful introduction of MDT so far has been achieved mainly in those countries or areas where the conditions are relatively 'easy' for the implementation of MDT: countries or regions with a good infrastructure or with sufficient numbers of well-trained health workers, a good coverage with health services, or with a pre-existing, well-managed leprosy control programme based on dapsone monotherapy, adequate financial resources, etc.

A lot has to be done in order to achieve and to sustain full MDT coverage. Unless the obstacles are really insurmountable, such as war situations, all known cases must be submitted to MDT within the next 3 to 5 years. We have to rationalize the leprosy control strategy in order to achieve this goal and to maintain adequate and appropriate services. The MDT coverage of registered cases is often taken as the only measure for progress in MDT implementation. This is wrong. We must also look at the coverage of the real case load and the cure rate (MDT completion).

WHO and ILEP publications have called for increasing attention to the basic rather than the optimal requirements to enable programme managers to achieve full MDT coverage. Further progress has been made with the acceptance of the essential indicators which should reduce the data requirements, the recent publication and the distribution of the ILEP document on guidelines for programme managers for the prevention of disability and the introduction of the WHO modules on training of programme managers in leprosy control.

Even more simplification and flexibility will be required for universal MDT implementation and to sustain it under low endemic conditions. Creative local approaches, sometimes specific for individual patients, will have to be developed by local health staff.

Recommendations for further simplication of guidelines for programme managers

HEALTH EDUCATION, AWARENESS AND STIGMA

Managers should encourage the widespread use of the mass media for promoting early case presentation. Groups in the community, particularly patients and ex-patients, can be utilized to support leprosy control services, promote case-finding, case-holding and

prevention of disability. Despite this and the widespread use of MDT, stigma is still a major problem in many areas and is even a problem within the health and medical professions. Integration of the leprosy services, more effective health education to the population and improvement of the socioeconomic status of patients, particularly those with disability, will assist in diminishing stigma.

CASE-FINDING, DIAGNOSIS AND CLASSIFICATION

Passive case-finding is the mainstay of new patient detection. This voluntary reporting should be supported by health education and awareness programmes, especially using the mass media. Good quality services, presented in a user friendly manner, should promote early case detection. Furthermore, contact examination should be prompt and should be stimulated by the newly-diagnosed leprosy patient. It does not have to be done repeatedly.

Diagnosis should be made at the peripheral health unit and must result in the immediate start of MDT. Only the PB/MB classification should be used in the field. The hoped-for adoption of a single agreed drug regimen will enable classification in the field to be finally abandoned. The presence of slit-skin smear services are not a pre-requisite for the implementation of MDT and these services do not need to be developed at the peripheral level. While there is still a role for skin smear microscopy at the referral centre level, histopathology services and the lepromin test have no relevance in routine leprosy control.

CASE-HOLDING

Services to patients must be flexible and encourage regularity of attendance. The fixed drug treatment regimens of 6 and 24 months should be adopted forthwith. Supervised intake of the once monthly pulse is still advocated but this does not necessarily require fixed monthly clinic days. When necessary patients or their relatives can be given several months of MDT. The use of blister packs is indicated in these circumstances.

In view of the low relapse rates, no active post-cure surveillance is indicated. Following adequate health education, it is the responsibility of the patient to report promptly any adverse developments, including any nerve function loss.

REFERRAL SERVICES, PREVENTION OF DISABILITIES (POD) AND REHABILITATION

Effective POD is not only for the benefit of the patients concerned, but also for the credibility of the programme. Increased credibility results in earlier self-reporting of new cases and leads to better treatment compliance. As such, POD will contribute to the elimination of the disease. Secondary referral services must be available. POD activities should be an integral part of the job description of the primary worker at the peripheral health unit. The simplest possible, reliable, method to identify new nerve function loss should be used, including asking the patient; and a quick VMT/ST should be able to be performed by all health workers dealing with patients. The earliest interventions for POD (reactions and neuritis) are the priority but, where circumstances permit, rehabilitation services should be made available, preferably as a part of the national rehabilitation services for the disabled, from whatever cause.

INTEGRATION AND COMBINATION

Vertical programmes hold clinics only periodically (monthly) and are often associated with the stigma of leprosy. As such they hinder an optimal relationship between the leprosy services and the community. Poor accessibility and acceptability result in delayed case detection and reduced compliance with chemotherapy. It is obvious that the general health services, which usually are closer to the community, permanently accessible and more acceptable must be involved in the treatment and retrieval of patients. The peripheral general health services staff should be aware of, should feel responsible for and should be involved in the management of leprosy. Integration does not mean that specialized services disappear, but rather that they should be available at a higher level, possibly in conjunction with other referral services.

While the support of technical expertise at national, regional and district level is required, a large work force of peripherally based technical staff is not likely to be cost-effective, especially in low prevalence areas. Co-operation with other vertical programmes may, in these circumstances, be more cost-effective and the chosen combination (TB, TB and chest diseases, tropical dermatoses, prevention of blindness, etc.) will depend on the local situation.

TRAINING

Multipurpose health workers should receive training in leprosy in their basic training curriculum. In addition, those posted in the field should have adequate, appropriate training followed by post-training supervision, suitable for their job description. All training should conform to the nationally agreed curricula. Training, including training in management, implies that the trainees are appropriately posted and can carry out the duties expected of them. Training of district level managers should also include the provision of more patient oriented services and in dealing effectively with the media.

MOTIVATION OF STAFF

Maintenance of a high quality of work performance is often difficult in the field. Job satisfaction is provided by enabling staff to do the work they are trained for, by providing the necessary facilities and drugs and by having supportive, regular supervision. Feed-back to peripheral staff on their performance and the progress of the programme will promote the feeling of direct involvement. Financial security does not necessarily require special incentives but a realistic salary and allowances should be paid promptly. A career structure is a necessity.

MONITORING AND EVALUATION

Despite the general adoption of the 6 essential indicators of leprosy control, there should be no pre-requisite that all or any of these indicators are in place before the implementation of MDT. The most essential indicators which should be available are the number of patients newly diagnosed and the proportion of these patients cured.

Workshop 12 Elimination of Leprosy

Chair: S. K. Noordeen
Rapporteur: Robert R. Jacobson

Participants

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The success of the multidrug therapy (MDT) regimens for the treatment of leprosy developed by a WHO study group in 1981 prompted the World Health Assembly to pass a resolution in 1991 on the elimination of leprosy. Through this resolution the WHO declared its commitment to continue to promote the use of all control measures, including multidrug therapy, together with case-finding, in order to attain the global elimination of leprosy as a public health problem by the year 2000. Elimination is defined as the reduction of prevalence to a level below 1 case per 10,000 population. In 1990, the International Federation of Anti-Leprosy Associations (ILEP) had adopted the target of MDT for all by the year 2000.

Achieving this goal through improved control efforts and provision of MDT for all leprosy cases does not mean complete eradication of the disease. It must, however, be considered the first essential step toward eventual eradication. It will lead to a situation where the disease is no longer a public health problem, i.e., where transmission of infection is expected to be drastically reduced.

New cases will still occur, since the fall in incidence to be expected if transmission has been interrupted may lag many years behind the fall in prevalence, and this fall depends on the early introduction of MDT with sustained high coverage. This, consequently, will also lead to reduced occurrence of disabilities.

Since the elimination strategy was adopted, progress with the widespread implementation of MDT has continued. By mid-1993, over 4·1 million patients had been cured with relapse rates of less than 1% overall. Likewise, the global current and cumulative MDT coverage of registered patients has reached 48% and 82%, respectively, with the number of registered cases reduced from 5·4 million in 1985 to 2·3 million now. Thus, the goal is achievable and presents a unique opportunity in the history of this disease. Its achievement, however, will require a continued major effort on the part of the endemic countries, the WHO and non-governmental organizations (NGOs) in order to increase MDT coverage as rapidly as possible.

Strategy

The first priority must be to treat rapidly all registered cases with MDT and improve case-finding in terms of coverage and early detection. Strong political commitment and collaboration between national governments, WHO and national and international NGOs and other donor agencies requires further strengthening to ensure the availability

of the necessary resources. National plans of action are a must which will guide activities. Resources must be mobilized to assure, among other things, adequate long-term supplies of drugs and equipment. Appropriate organization of the existing leprosy services, whether vertical or integrated, and updating of existing information by clearing the registries to accurately identify the number of cases needing MDT will then allow proper implementation of the plan.

The elimination strategy aims to stratify the situation at different levels, identify priority areas for action, set intermediate targets and monitor them. Such an approach, however, should not neglect areas of low prevalence within countries or countries with a low prevalence. This requires review of the situation country by country and, within each country, area by area.

Improved case detection is important to the success of the programme. Self-reporting of suspect lesions is the most cost effective approach. Training of peripheral health services to recognize the disease is also vital. Self-reporting can be encouraged through the use of volunteers and community workers and through innovative use of the media to increase community awareness of the problem and reduce the stigma.

Regular monitoring and evaluation of the programme is essential to see that progress continues toward specific targets, and problems are identified early. Health systems research may be helpful to identify and solve problems early.

In spite of widespread implementation of MDT and its contribution to prevention of disabilities, there will remain a significant number of persons either with or at risk of disability who will require care and rehabilitation.

Constraints

Bringing MDT to the remaining patients still poses a considerable challenge. In existing programmes an effective infrastructure will need to be maintained, but seeing fewer patients. This would mean increasing cost per patient. Furthermore, many patients will be in areas that are operationally difficult due to geography, infrastructure or civil disturbance.

As prevalence decreases, increased efforts to maintain the political commitment for the programme will be necessary as the needs of leprosy control may be considered in relation to the needs of other health problems and health policy in general. Programme planning must now look beyond attainment of the elimination to the maintenance of the necessary skills to detect cases where the prevalence becomes very low. This may be accomplished by training personnel to suspect leprosy whenever appropriate and the maintenance of a core area of expertise to confirm the diagnosis of cases on referral and manage complicated cases. In this and in the urban control efforts, the private medical sector may play a significant role in certain countries provided they follow the national guidelines regarding classification and therapy.

Existing cases requiring MDT and cases expected to be detected between now and the year 2000 may total as many as 6 to 7 million patients. It is estimated that 400–500 million USA dollars will be required for this effort. Thus, the continued commitment of national governments, NGOs and the WHO until the year 2000 and beyond is vital if this effort is to succeed.

Research

Current research efforts may yield shorter-term therapy and/or fully supervisable therapy for those cases who require other than standard MDT. These would accelerate attainment of the elimination goal. Other areas of research should include improved anti-reaction therapy and prevention of nerve damage.

Conclusion

Intense efforts on the part of all involved are required to eliminate leprosy as a public health problem by the year 2000. The basic resources and technology exist. It would be inexcusable if the efforts are not made and this historical opportunity is missed.

Workshop 13 The Eye

Chair: Dr Felix Brandt
Rapporteur: Dr Timothy ffytche

Participants:

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Ebenezer Daniel
Tafessawork Girma
Margaret Hogeweg
Mary Jacob
Murat Karacorlu
Wiebe Jan Lubbers
M. A. Rajan
Swapan K. Samanta
Alberte Schipper
Shi Zhenrong
William J. Woods
Zhou Huiming
Elsbeth Zyp-Klaver

The meeting was opened by Dr Margaret Brand who reviewed the current situation of ocular leprosy, highlighting some of the problems of the disease facing workers in the field.

Following this there were 6 sessions on wideranging topics which included the prevention, cure and care of blindness in leprosy, ocular pathology in the disease, ocular surgery and ocular complications seen at presentation, during and after chemotherapy.

The final session was devoted to a discussion of the setting up of various projects to be undertaken by members of the group in anticipation of the next meeting. Due to lack of time several important subjects could not be addressed, these included epidemiology and the training of medical staff.

Summary of the main points of discussion

OCULAR SURVEYS

It was generally agreed that the value of horizontal surveys was limited, although important in drawing attention to the current ocular problems in the areas surveyed. It

was recommended that longitudinal surveys should be encouraged with the standardization of data wherever possible. Too often there are differing definitions of such important measurements as blindness and visual impairment, and the evaluation of clinical entities such as lagophthalmos, diminished corneal sensation and iris atrophy needs to be standardized.

OCULAR PATHOLOGY

The group recognized that there is still a great lack of pathological studies on tissues of the eyes of leprosy patients in all stages of ocular involvement, even when there is little or no evidence of this. It was recommended that specimens should be retained for histological examination and sent to ocular pathologists identified by the group. It was emphasized that wherever possible specimens should be accompanied by clinical data on the patient, and that the specimens should be fixed in 10% formalin, or, in the case of small biopsy tissues, in 2·5% glutaraldehyde if available. At autopsy, eyes and skin specimens taken from sites known to be affected should also be sent. This aspect of research was regarded as a high priority.

Immunological studies on ocular tissue should also be encouraged where the appropriate facilities for examination exist.

CLINICAL EXAMINATION

The group recommended that registry cards used in any leprosy programme should include a section dealing with the eyes, and that training manuals for leprosy workers and eye workers should give more attention to eye care and the prevention of ocular complications.

On more specific points the group agreed on a definition of blindness to be used in future surveys: 'Blindness' was defined as corrected vision of less that 3/60 in the better eye. Vision less than 6/60 in the better eye was termed 'severe visual impairment'.

There was a long discussion on facial nerve involvement, including muscle weakness, lagophthalmos and exposure. It was admitted that the current classification of this condition was unsatisfactory and the group spent a great deal of time on grading the clinical examination of the condition. It was agreed that lagophthalmos should be graded as:

1 normal;
2 orbicularis muscle weakness;
3 lid gap with cornea cover in mild closure;
4 lid gap with cornea exposed in mild closure.

It was generally agreed that impairment of corneal sensation is one of the most important factors in the production of eye complications in leprosy. Quantitative measurements remain difficult and the traditional method of testing with a cotton wool wisp is probably the best—3 levels of sensation can be recorded in this way: normal, diminished and absent, although grading diminished sensation can be a problem. It is recommended that corneal sensation is tested by touching the centre of the cornea, and this should be carried out routinely by paramedical workers.

There were several presentations on iris atrophy and its early diagnosis. It was

suggested that measurement of the pupil/cycle time (PCT) would be an interesting clinical examination in early cases.

It was generally agreed that treatment carried out at the early stage of the disease reduces the incidence of ocular complications. But evidence was presented showing that problems can exist at the time of diagnosis and also arise during treatment. An important finding was that a significant proportion of patients released from treatment (RFT) had sight-threatening lesions requiring continued follow-up and management, and there is evidence that new ocular problems due to leprosy can occur in patients classified as 'cured'.

Although the results of intraocular surgery, particularly cataract removal, are not as bad as expected, there is room for improvement and the introduction of intraocular lenses will add a new dimension which will need to be carefully considered. This subject was discussed at length and recommendations were made for future comparative studies of different types of cataract surgery.

PROJECTS SET UP

It was agreed the following projects should be set up in advance of the next ILA meeting:

(a) pathology scheme. Supervisor—F. Brandt;
(b) cataract study. Supervisor—M. Rajan;
(c) IOL study. Supervisor—M. Karacorlu;
(d) chemotherapy. Supervisor—M. Rajan;
(e) pupil/cycle time. Supervisor—M. Karacorlu.

Final summary

The group expressed their gratitude to the ILA for making this workshop possible and drawing attention to the ocular complications of leprosy. It was noted that many participants who planned to attend were unable to because of financial restrictions and difficulties in obtaining visas. It is to be hoped that the ILA will be able to overcome these problems when the next workshop is organized.

The group congratulated Felix Brandt on his organization of an excellent meeting despite the difficult circumstances.

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Errata

Attitudes towards leprosy in the outpatient population of dermatology clinics in Trinidad. M. Suite and C. Gittens. *Lepr Rev* (1992) **63,** 151–156.

Page 155, line 11 onwards should read: '. . . effect 64 respondents (70%) claimed that they would be supportive of friends and relatives who contracted leprosy but only about half of those expected support from others if they had the disease.'

– – – – –, line 14 should read: 'It is our experience that a common response to the diagnosis is that patients question how they contracted . . .', i.e. the *patients* are the ones who want to know from where their disease originates.

Letter to the Editor: Disappointing experiences with blister-calendar packs. P. Lever. *Lepr Rev* (1993) **64,** 171.

The name of Dr Hudion Boutmy, who is co-author of the above Letter, was omitted at the time of publication.