Images from the History of Leprosy

An overview of the Schieffelin Leprosy Research & Training Centre, Karigiri, Tamil Nadu, India, photographed in 1962. SLR&TC, better known as “Karigiri,” was established 50 years ago, and quickly established an international reputation as a center of training and research on medical, surgical, and laboratory aspects of leprosy. SLR&TC remains an active collaborating center in leprosy research (see News & Notes).

This photograph is part of a collection of historic photographs given to SLR&TC by the Schieffelin family. Taken in 1962 by Christa Pohlschroder Ficht, this is a digital copy of an original color print measuring 3 × 3 inches. The photograph and information are courtesy of SLR&TC, Barbara Schieffelin Powell & Mr. Justin Bossanquet-Rossen.
Analysis of 6000 Skin Biopsies of the National Leprosy Control Program in Mexico

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ABSTRACT

Six thousand skin biopsy specimens taken from April 1978 to January 2002 under conditions as specified by the National Leprosy Control Program (NLCP), were analyzed to obtain information about the work of the program and contribute to the knowledge of this illness in the Mexico. Six-thousand request forms for histologic exam of the NLCP were reviewed. Sixty-two percent of the requests had all the required information and in 38% one or more data items were omitted. The age range was 2 to 98 yrs with a median of 50 yrs; a small number of cases was observed in the age group of 0 to 14 yrs, and the peak was in the age group of 41 to 50 yrs. Of the 6000 biopsies, 3693 were classified. Polar lepromatous (LL) was the most common form of the disease, in 60.3% of cases. Twice as many cases were multibacillary leprosy (MB) as paucibacillary (PB). MB predominated in males, and PB predominated in females. The Cohen’s kappa index ($\kappa$) of clinical-histological agreement was 0.202 (95% CI 0.184–0.219) and showed a poor grade of agreement between clinical and histologic diagnosis, with a level of significance of 0.05 ($p < 0.001$). The results may indicate the end of leprosy in Mexico, a country in which the national goal of elimination was reached in 1994, with a prevalence since the year 2000 of 0.17/10 000.

RÉSUMÉ

Six mille biopsies cutanées prélevées d’Avril 1978 à Janvier 2002, selon les spécifications du Programme National de Contrôle de la Lèpre (PNCL) furent analysées et traitées afin d’obtenir plus d’informations sur le travail de ce programme et à sa contribution à la connaissance de cette maladie au Mexique. Six mille demandes d’analyse histologique du PNCL furent revues. Soixante deux pour cent des requêtes présentaient tous les renseignements tandis que 38% de celles-ci avaient une ou plusieurs données manquantes. L’intervalle d’âge était de 2 à 98 ans avec un médian de 50 ans; un faible nombre de cas fut observé dans la classe d’âge de 0 à 14 ans, et le pic de demandes était parmi les 41–50 ans. De ces 6000 biopsies, 3693 furent classées. La lèpre lépromateuse polaire fut la forme la plus fréquente de la maladie avec 60.3% des cas. Il y avait deux fois plus de cas de lèpre multibacillaire (MB) que de lèpre paucibacillaire (PB). La lèpre MB dominait chez les patients de sexe masculins, tandis que la lèpre PB était plus fréquente chez les patientes. L’index kappa ($\kappa$) de Cohen de concordance clinico-pathologique était de 0.202 (IdC à 95% de 0.184 à
RESUMEN

Se analizaron seis mil biopsias de piel tomadas de Abril de 1987 a Enero de 2002 bajo las condiciones especificadas por el Programa Nacional de Control de la Lepra (PNCL) para obtener información sobre el trabajo del Programa y para contribuir al conocimiento de esta enfermedad en México. Se revisaron 6,000 solicitudes del PNCL para realizar el examen histológico de las biopsias. Sesenta y dos porcentaje de las solicitudes contenían toda la información requerida, mientras que en el 38% restante se habían omitido uno o más datos. El rango de edad fue de 2 a 98 años, con una media de 50 años; en el grupo de 0 a 14 años se observó un bajo número de casos, y el pico estuvo en el grupo de 41 a 50 años. De las 6000 biopsias, 3693 pudieron ser clasificadas. La lepra lepromatosa polar fue la más común de las enfermedades, con 60.3% de los casos. Los casos multibacilares (MB) fueron el doble de los paucibacilares (PB). Los casos MB predominaron en los hombres mientras que los PB predominaron en las mujeres. El índice kappa de Cohen (κ) de congruencia clínico-histológica fue 0.202 (95% CI 0.184–0.219) y mostró una fuerza de asociación pobre, con un nivel de significancia de 0.05 (p <0.001). Los resultados podrían indicar el final de la lepra en México, un país en el cual la meta nacional de eliminación de la lepra se alcanzó en 1994, con una prevalencia de 0.17/10,000 desde el año 2000.

MATERIALS AND METHODS

This report describes the analyses of 6000 biopsies obtained from cutaneous lesions of subjects suspected to have clinical leprosy when the medical staff of the National Leprosy Control Program (NLCP) examined them. In April 1978, the Laboratory of Dermatopathology of the Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), formerly Instituto de Salubridad y Enfermedades Tropicales, started the diagnosis and histopathologic classification of skin biopsies of the NLCP, and from that date to January 2002, 6000 skin specimens were received for microscopic diagnosis and histologic classification.

This study was designed to provide information about the work of the program and contribute to the knowledge of this illness in Mexico. With this purpose, the following secondary objectives were established: to determine the duration of the disease before subjects sought medical care at NLCP; to identify the errors that influenced the result of the biopsy; to correlate the topography and type of the lesions with the histologic classification; to specify the frequency of leprosy by age and gender in the country; to define the histologic spectrum of leprosy in Mexico and to determine the agreement between clinical and histologic diagnosis in skin biopsies of the NLCP.

Six thousand skin biopsy specimens and 6000 request forms, submitted from April 1978 to January 2002 for diagnosis and histologic classification of the NLCP, were reviewed and analyzed to determine: (i) duration of the disease; (ii) gender and age of the subjects suspected to have leprosy; (iii) clinical diagnosis; (iv) biopsy result; (v) confirmation of the clinical diagnosis for biopsy; (vi) characteristics of the type and site of lesion selected for biopsy; (vii) Errors that hindered the histologic examination.

All biopsy specimens were obtained from subjects suspected to have clinical leprosy, and dispatched in glass or plastic vials containing 10% formalin solution from different provinces of the country to the InDRE in Mexico City where all histologic processing was carried out. Specimens were embedded in paraffin and sections of 8 µm were obtained. They were stained with hematoxilin-eosin and with the Fite-Faraco modification of the Ziehl-Neelsen stain for acid-fast bacilli. Ten to 20 sections were cut from each block and the search for bacilli was done in various sections. All microscopic studies were made by one dermatohistopathologist. The histologic criteria for
diagnosis and classification of leprosy for tuberculoid, borderline tuberculoid, borderline lepromatous, and lepromatous, were those described by Ridley and Jopling (11, 12):

**Tuberculoid leprosy** (TT). Epithelioid cell granulomas with Langhans giant cells surrounded by dense lymphocytic infiltrate, erosion of epidermis and infiltration of the nerves; acid-fast bacilli (AFB) zero.

**Borderline tuberculoid** (BT). Epithelioid cell granulomas with peripheral lymphocytes and Langhans giant cell, with a clear subepidermal zone and infiltration of the nerves; AFB 0 to 2+.

**Borderline** (BB). Epithelioid cells granulomas with diffusely spread lymphocytes, presence of a subepidermal clear zone and nerve bundles recognizable; AFB 3 to 4+.

**Borderline lepromatous** (BL). Granuloma composed of histiocytic cells that show a tendency to epithelioid cells and sometimes to foamy change, with areas of dense lymphocyte infiltration as perineural cuffs or occupying a whole segment of the granuloma. Large globi absent. AFB 4 to 5+.

**Lepromatous leprosy** (LL). Granulomas composed of histiocytes and foamy cells and, eventually, globi. Lymphocytes are scanty: if present they are diffusely spread. Nerves are without cellular infiltration or cuffing. There is a clear Grenz zone; AFB 5 to 6+.

**Intermediate** (I). Lymphocytes and histiocytes localized around skin structures. Acid-fast bacilli are very scanty in all cases.

**Diffuse leprosy** (DLL). An unusual variety of the lepromatous type of the disease, this was first described by Lucio and Alvarado in 1851 in Mexico (7). Clinically, this is characterized by a diffuse infiltration of the skin over the entire body. The skin may appear normal on clinical inspection but *Mycobacterium leprae* are usually obtained by scraping from any region of the skin. Histologically there is an infiltrate with foam cells that surrounds the blood vessels. From the surface downward the density of the cellular infiltrate increases; it is slight to moderate around the blood vessels of the superficial plexus and denser with tendency to form small nodules around the blood vessels of the deep plexus. The most conspicuous feature is the presence of innumerable lepra bacilli within the endothelial cells of small vessels of the papillary and subpapillary dermis, and within the endothelial cells and muscular layer of the larger vessels of the deeper dermis and subcutaneous fat; AFB 6+. (These histologic criteria were based on personal observations not previously published).

**Histoid leprosy** (HLL). Nodules characteristically present over apparently normal skin. Histologically the lesions show circumscribed histoid lepromas characterized by the predominance of spindle-shaped histiocytes containing acid-fast bacilli; AFB 6+ (15).

The clinical diagnosis was made according to Madrid classification (9), which considers two polar types: lepromatous (L) and tuberculoid (T); and two groups: indeterminate (I) and dimorphous (D). And it includes the term variety as a subdivision of type or of group. Type includes the cases, defined as stable and invariable. Group includes the unstable cases, mutable, and of uncertain evolution. Clinically lepromatous is characterized by nodules, plaques, and macules. Tuberculoid is characterized by plaques, macules, and annular lesions. Dimorphous is a characterized by a great variety of lesions with characteristics of L and T. Indeterminate is characterized by hypopigmented macules with dysesthesia, anhidrosis, and alopecia (DAA).

Statistical significance was determined using the test for differences between proportions; the numbers of males and females at each interval were compared by Kolmogorov-Smirnov test, and the Cohen’s Index kappa was used to determine the correlation coefficient between clinical and histologic diagnosis (2).

**RESULTS**

Of the 6000 biopsy specimens of the skin from subjects suspected to have leprosy, 3693 (61.6%) were classified, 2010 (33.5%) did not show histological evidence of leprosy, and 297 (4.9%) were not included in this study, 86 because they were inadequate specimens, and 211 because they were specimens taken from treated subjects. Of the 2010 unclassified cases, 1463 showed nonspecific features, 358 had the histologic appearance of normal skin and 189 exhibited findings of other dermatosis different from leprosy (Fig. 1).
Duration of the disease before medical staff of the National Leprosy Control Program examined subjects. Duration of the disease before medical staff of the control program clinically examined the subjects ranged from one month to 60 yrs, with a median of 18 months Of the 3693 classified biopsies, in 7% of the requests the date of onset was omitted, 13% of the patients were examined from one to 12 months after onset, 30% after one to three years, 22% after 3 to 5 yrs, 13% after 5 to 10 yrs, 9% after from 10 to 20 yrs, 3% after 20 to 40 yrs, one after 55 yrs and one patient 60 yrs after onset.

The relationship between the form of the leprosy and the duration of the disease was: for indeterminate, from one month to 25 yrs with a median of one year; for tuberculoid, from two months to 30 yrs with a median of 2 yrs; for borderline, from 45 days to 20 yrs with a median of 9 months; and for lepromatous, from two months to 60 yrs with a median of 3 yrs.

Common errors that rendered the biopsy specimen histologically unreadable. Most of the biopsy specimens of the skin were fusiform obtained with a scalpel, and contained subcutaneous fat tissue, but in 86 (1.4%) of the 6000 biopsy specimens, there were errors that rendered the pieces of tissue histologically unreadable. Of these, 51 were superficial and insufficient, consisting only of epidermis, without bacilli or inflammation, 24 were not well fixed (some of them were placed in distilled water), 7 specimens were received dry (six inside of an empty broken glass vial and one without vial), and 4 vials were received without a specimen (Fig. 2).

The anatomic site from which the biopsy specimens were obtained and classified. Forty percent (1461/3693) of the skin specimens were taken from the upper extremity and 33% (1203/3693) from the lower extremity. These specimens were mostly taken from the forearm (732/1461) and arm (562/1461); and from the leg (596/1203) and thigh (395/1203). However, the only site that provided the most precise information and with the best percentage of histological agreement were the specimens
taken from the ear; in 59% of them the diagnosis of LL was confirmed (Fig. 3).

**Correlation between the type of the clinical skin lesion and classification.** Macules, nodules, and plaques were the skin lesions most frequently biopsed (Fig. 3). In 38% (1392/3693) of the classified cases, a macule was the skin lesion selected for biopsy and the histologic features most frequently seen in this lesion were those of I, TT, and BT. Nodules accounted for 21% (779/3693) of the lesions biopsied. This was the only skin lesion that had high agreement with the biopsy result, 82% of the nodules showing histologic evidence of LL. Plaques were selected in 16% (578/3693) of the classified cases and the histologic signs of TT and BT were more commonly present in these biopsies.

The correlation between the skin lesion and histologic diagnosis showed the following results. In the indeterminate type, macules and skin with DAA were the most common skin lesions biopsed. In TT and dimorphous groups, macules and plaques were the most selected. In LL, nodules, macules, and plaques were observed, and in DLL, skin with DAA and macules were most common. Finally, in HLL, a nodule was most commonly biopsed.

**Distribution of 3693 classified cases, by age and gender.** Table 1 shows the age and gender distribution of all 3693 classified cases; 61.3% were male and 38.7% female, with a male-to-female ratio (M:F) of 1.6:1. The age range was 2 to 98 yrs, with a median of 50 yrs; 4.7% were children in the age group of 2 to 14 yrs and the greatest percentage (19.2%) was seen in the age group of 41 to 50 yrs. When the frequency of ages of males and females was compared, by decade, there were no significant differences in their distribution (p <0.3) (Fig. 5).

The results of 3693 classified cases by histological examination are shown in Table 1. Four hundred eighty-eight (13.2%) cases revealed features of I, 316 (8.6%) of TT, 411 (11.1%) of BT, 153 (4.1%) of BB, 98 (2.7%) of BL, 2042 (55.3%) of LL, 174 (4.7%) of DLL and 11 (0.3%) of HLL. LL was much more frequent in males (68.0%) than in females (32.0%), with M:F ratio of 2.1:1 (p <0.0001). TT was the only form of leprosy more frequent in females (36.4%) than in males (36.4%), with F:M ratio of 1.7:1 (p <0.0001); both differences are statistically highly significant. Multibacillary leprosy (MB) with 67.9% of the total, was twice as frequent as paucibacillary leprosy (PB), with 32.1% of the cases, and involved more males than females 2.1:1. PB was more common in girls (25/43) than in boys (18/43) in the age group 2 to 10 yrs old, and in women (400/669) than in men (269/669) in the age group

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**Fig. 3.** Correlation between the site where the biopsy was taken and its histologic diagnosis.
In 174 children aged 2 to 14 yrs, a histologic diagnosis of lepromatous leprosy (including LL, DLL, and HLL) was made in 39.1% (63) of the cases, I in 25.9% (45), TT in 17.2% (30), BT in 11.5% (20), BL in 3.5% (6), and BB in 2.9% (5) (Table 1).

Agreement between clinical and histologic diagnosis. In 5199 biopsy specimens were examined histologically and the findings were correlated with their clinical diagnosis (Table 2). There was complete agreement between the clinical and histologic diagnosis in 42.9% of the cases (Fig. 5). We employing the Cohen’s Kappa index ($\kappa$) and the 95% confidence intervals (CI) around kappa to evaluate the statistical significance of agreement between clinical and histologic diagnosis, $\kappa = 0.202$ with a 95% CI of 0.184 to 0.219 with a level of significance of 0.05 $p < 0.01$. The kappa index of agreement of the clinical diagnosis and the histopathologic diagnosis for the different forms of leprosy, and the 95% confidence interval for $\kappa$ are seen in Table 2 and Figure 6.

In the correlation of the clinical and histologic diagnosis, the agreement in the dimorphous group was not analyzed in its different varieties because the physicians of the control program, in accordance with the Madrid classification, only made a clinical diagnosis of dimorphous, and the histologic diagnosis was according Ridley and Jopling criteria.
DISCUSSION

This is a retrospective study of biopsies that have been routinely collected over a period of nearly 24 yrs from all cases suspected to have leprosy in a national control program. The main aims of this retrospective analysis were to try to obtain information on the work of the program, and to contribute to the knowledge of leprosy in the country.

This study was implemented under routine program conditions and with numerous limitations. Nonetheless, the data can be regarded as representative of the population of leprosy patients in general, and there is sufficient information to draw some conclusions.

The time of duration of disease before medical staff of the program diagnosed leprosy clinically, varied from one month to 60 yrs with a median of 18 months. The 44% of the cases were from one month to three years, 22% was from three to five years, and 30% was from five to 40 yrs. In various countries the majority of new cases detected have a delay of one to three years, and WHO collaborative studies indicate that the majority of new cases are detected late; i.e., that about 75% of new cases are

TABLE 2. Concordance correlation coefficient (Cohen’s kappa index) between clinical and histologic diagnosis.

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>I</th>
<th>TT</th>
<th>BT</th>
<th>BB</th>
<th>BL</th>
<th>LL</th>
<th>DLL</th>
<th>HLL</th>
<th>NCI</th>
<th>NS</th>
<th>NO</th>
<th>Total</th>
<th>κ Index</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indeterminate</td>
<td>307</td>
<td>80</td>
<td>63</td>
<td>11</td>
<td>4</td>
<td>204</td>
<td>7</td>
<td>665</td>
<td>158</td>
<td>47</td>
<td>1546</td>
<td>0.205</td>
<td>0.187–0.223</td>
<td></td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>51</td>
<td>168</td>
<td>85</td>
<td>9</td>
<td>4</td>
<td>61</td>
<td>1</td>
<td>185</td>
<td>46</td>
<td>12</td>
<td>622</td>
<td>0.323</td>
<td>0.306–0.341</td>
<td></td>
</tr>
<tr>
<td>Dimorphous**</td>
<td>14</td>
<td>15</td>
<td>159</td>
<td>60</td>
<td>29</td>
<td>80</td>
<td>4</td>
<td>84</td>
<td>16</td>
<td>12</td>
<td>473</td>
<td>0.409</td>
<td>0.391–0.427</td>
<td></td>
</tr>
<tr>
<td>Lepromatous</td>
<td>71</td>
<td>22</td>
<td>75</td>
<td>54</td>
<td>25</td>
<td>1432</td>
<td>86</td>
<td>11</td>
<td>292</td>
<td>92</td>
<td>81</td>
<td>2241</td>
<td>0.478</td>
<td>0.458–0.498</td>
</tr>
<tr>
<td>Diffuse lepra</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>155</td>
<td>74</td>
<td>59</td>
<td>12</td>
<td>1</td>
<td>317</td>
<td>0.271</td>
<td>0.254–0.289</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>443</td>
<td>293</td>
<td>385</td>
<td>138</td>
<td>63</td>
<td>1932</td>
<td>172</td>
<td>11</td>
<td>1285</td>
<td>324</td>
<td>153</td>
<td>5199</td>
<td>0.202</td>
<td>0.184–0.219</td>
</tr>
</tbody>
</table>

I = Indeterminate, T = Tuberculoid, BT = Borderline tuberculoid, BB = Borderline, BL = Borderline lepromatous, LL = Lepromatous, DLL = Diffuse leprosy, HLL = Histoid leprosy, NCI = Nonspecific chronic inflammation, NS = Skin apparently normal, NO = Other diagnostic different from leprosy.

* CI = Confidence interval.

** Dimorphous = Includes BT, BB, BL.

This table does not include 801 cases (504 without clinic diagnosis, 211 from subjects treated and 86 inadequate specimens).
detected 3 to 5 yrs after onset and about 15% are detected 5 to 10 yrs after the onset of the disease.

Of the skin biopsy specimens received, 86 (1.4%) could not be studied due to faults in the sampling, obtaining, fixation, or dispatch. Thus, it is necessary that clinicians be acquainted with the importance of proper selection of the site and type of lesion for histologic examination, as well as the correct methods for fixing the piece of tissue and packing and mailing it to the laboratory.

The preferred anatomic sites of the skin biopsy specimens were the forearm (20%) and the leg (16%), but the anatomical site with best percentage of positive clinico-histologic correlation was the ear, where 56% of the specimens confirmed the diagnosis of LL. Macules were the lesions most frequently biopsed, and in these skin lesions the histologic features of I, TT, and BT were more frequently found. The nodule was the most significant lesion for LL.

MB (67%) occurred twice as frequently as PB (33%) and affected more males than females (2:1), but TT was more common in females than in males. This agrees with most studies throughout the world that indicate that males are approximately twice as likely to contract lepromatous disease as are females.

According to these results, LL was by far the most common form of leprosy encountered in Mexico, with 60.3% (2227/3693) of cases. In Mexico the diffuse lepromatosis is more frequent. This is the most severe of all forms of leprosy and its striking histologic feature is the presence of innumerable lepra bacilli within the endothelial cells of the capillary vessels of the papillary and subpapillary dermis. These results indicate that a high percentage of the population of this country has a very low degree of resistance to this infection.

It is interesting to note that most previous authors have recorded a higher frequency rate in children (<14 yrs old), and that LL is infrequently seen in this group (3,6,14). However, in this study the frequency in children was low (4.7%), and the most common form of the disease in this age group was LL; it is also of note that in infants and children under 6 yrs old, TT was most common in girls and LL in boys.

The number of patients was greater in the 21 to 30 yrs age group, and the peak was reached in the 41 to 50 yrs age group, and declines in females aged 51 to 60 yrs, and

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**Fig. 6.** Correlation between clinical and histological classification of 3693 biopsies.
in males aged 61 to 70 yrs. These data could indicate that we are facing the end of leprosy in Mexico, a country in which the national goal of elimination was reached in 1994, with a prevalence since the year 2000 of 0.17/10,000. However, areas persist in which the elimination has not been achieved and they require us to persevere with continuous efforts to achieve elimination in the whole country, to cut the transmission of the disease and in the future to achieve its eradication.

There was complete agreement between the clinical and histologic diagnosis in 42.9% of the cases in this study. Other authors have reported percentages of complete agreement between clinical and histologic diagnosis in 33% to 89% of the cases (3, 4, 5, 9, 10, 13). The highest percent of agreement for individual types in this analysis was for LL (in 63% of the cases), and the lowest in I (20% of the cases). Agreement in the dimorphous group was not analyzed because the physicians of the control program, in accordance with classification from Madrid, only made a clinical diagnosis of dimorphous.

The concordance correlation coefficient between clinical and histologic diagnosis was determined using the index kappa of Cohen and was found $\kappa = 0.20$, which is a poor grade of agreement. This grade of agreement and the low percentage obtained, indicate the importance of measuring the accuracy of the clinical diagnosis, an important part of medical training that may allow reduction of failures in this field, since the clinical diagnosis still remains the mainstay for the detection of leprosy.

One hundred and eighty-nine studies requested with the clinical diagnosis of leprosy showed histologic findings of other dermatosis different from leprosy. Of these, the majority were interpreted as being neurofibromatosis, atopic dermatitis, pityriasis alba and lipomas.

Finally, the biopsy in leprosy is essential for the proper diagnosis, classification and prognosis of the disease and assessment of progression or regression of the disease in patients under treatment (1). But the biopsy has its limitations, with relative frequency it cannot enable a definitive diagnosis, but can only be suggestive in tuberculoid and indeterminate forms. The pathologist can give to the clinician enough information if the specimen is obtained, handled, fixed, and mailed correctly, and if the clinician provides detailed information of the patient and the disease when submitting a specimen to the laboratory for diagnosis. In order to use this knowledge most efficiently, close communication between pathologist and clinician is essential for their own understanding of the disease process, and for the benefit of the patients.

Acknowledgment. Thanks are due to Sergio Pas-tén, Biologist, from Department of Parasitology, for his help in statistical analysis.
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The Dynamics of Stigma in Leprosy

M. L. Heijnders

ABSTRACT

Leprosy in Nepal is a stigmatizing disease. This paper explores the different coping strategies employed by people affected by leprosy to manage stigma. It is based on a qualitative study conducted in the eastern part of Nepal. It will show that a difference exists between experienced stigma and the anticipation of stigma. Both types of stigma result in different coping strategies. In managing stigma people go through different phases. This paper will show that stigma is a dynamic process, and I will elaborate on the concealment cycle, as developed by Hyland, to produce a more detailed understanding of the stigmatization process in Nepal. Doing so, it highlights the importance of a mutual concealment phase and the importance of triggers to exposure and discrimination. Changing from one phase to a subsequent phase in the stigmatization process is always triggered. It highlights further, that even within the same culture and even the same village, social differentiation makes a significant difference on the impact of stigma and the coping strategies employed in managing stigma. Stigma enforces already existing inequalities in social class, gender, and age.

RÉSUMÉ

La lèpre au Népal est une maladie stigmatisée. Cet article étudie les stratégies variées utilisées par les personnes souffrant de lèpre pour éviter la stigmatisation. Il utilise des données d’une étude qualitative conduite dans la partie Est du Népal. Il montre que des différences existent entre une stigmatisation déjà vécue et une stigmatisation anticipée. Les deux types de stigmatisation résultent en des stratégies différentes d’évitement. Pour éviter la stigmatisation, les personnes passent par plusieurs phases. Cet article va démontrer que la stigmatisation est un processus dynamique, et je vais m’étendre plus particulièrement sur le cycle de dissimulation, comme développé par Hyland, afin de mieux comprendre et de façon plus détaillée le processus de stigmatisation au Népal. Ce faisant, cet article montre l’importance de la phase de dissimulation mutuelle et de l’importance des facteurs déclenchant lors de la révélation et de la discrimination. Le changement d’une phase à la suivante en stigmatisation est toujours déclenché. Cet article met de plus en lumière que, même au sein d’une même culture et dans le cercle d’un même village, la différenciation sociale introduit une différence significative sur l’impact de la stigmatisation et dans les stratégies d’évitement dirigées contre celle-ci. La stigmatisation met en application et fait valoir les inégalités déjà existantes de classe sociale, de genre et d’âge.

RESUMEN

La lepra en Nepal es una enfermedad estigmatizante. Este trabajo explora las diferentes estrategias seguidas por la gente afectada de lepra para sobreponerse al estigma. Se basa en un estudio cualitativo realizado en la parte oriental de Nepal. Muestra que existe una diferencia entre el estigma ya experimentado y la anticipación al estigma. Cada tipo de estigma se acompaña de diferentes estrategias de protección. Para manejar el estigma la gente pasa por diferentes etapas. El estudio muestra que el estigma es un proceso dinámico y en él se analiza el ciclo de confinamiento desarrollado por Hyland para entender de manera más detallada el proceso de estigmatización en Nepal. Al hacer esto, se resalta la importancia de una fase de confinamiento mutuo y la importancia de eventos que disparen la exposición y la discriminación. El cambio de una fase a la fase subsiguiente en el proceso de estigmatización es siempre un evento disparado. El estudio subraya además que dentro de la misma cultura y aún en la misma localidad, la segregación social influye de manera muy importante en el impacto del estigma y en las estrategias seguidas para manejarlo. El estigma refuerza las desigualdades ya existentes en clase social, género y edad.
Stigma is a dynamic process. Many people with leprosy are affected by stigmatization. For the individual, stigma often leads to a “spoiled identity” (6). After a person is labelled as leprous, there are negative social consequences for this person and his or her family. Stigma marks the possessor as socially unacceptable or as an inferior being, and the effect of “being labelled in this way is that people’s stigma can come to dominate the perception that others have of them and how they treat them” (12). Van Brakel, in his review on measurement of stigma, concluded that “despite enormous, cultural diversity, many areas of life affected by stigma are remarkably similar in different countries. They include mobility, interpersonal relationships, marriage, employment, leisure activities and attendance at social and religious functions” (14). However, how people are affected by stigma varies from culture to culture.

It is important to regard stigmatization as a process (9, 11, 16). During this process the label or attribute given to a person influences the social interaction between the labelled person and others. The person is labelled negatively, his or her status is devalued, and consequently the label could have negative consequences for this person, personally and socially. As Hayward and Bright (7), and Bainson and van de Borne (2) argue, the label modifies the actions of others towards the labelled person. Bainson and van de Borne further argue that probably the often present physical marks in leprosy generate a negative response in other people. According to Goffman (6), persons who share the same cause of stigma tend to have similar learning experiences in the management of the stigma, and they go through the same socialization process. He conceptualizes this as “the moral career of stigma.” The moral career is the sequence of adjustments people make in managing stigma. Similarly, Alonzo and Reynolds (1) and Hyland (9) describe stigmatization as a dynamic process, in which the affected person passes through several phases. In each phase, the person affected copes by using specific coping strategies and in each phase, other people’s responses are different.

The stigma trajectory as developed by Alonzo and Reynolds (1) shows how persons affected by stigma struggle with the expected stigma and how they cope with it. Scambler (12) differentiates between felt and enacted stigma. Felt stigma refers to the fear of being stigmatized and enacted stigma is the actual discrimination. In the stigma trajectory, people move from a felt stigma towards being actually stigmatized. The dynamics of the stigma trajectory is the result of the constant interaction between the person affected and other people (1). In the stigma trajectory, changing from one phase to the subsequent phases depends very much on the development of the disease, and in this, the development of visible symptoms. Additionally, characteristics of the individuals affected and the contextual situation can influence this process and the impact of stigma. Some people may have better coping skills or “passing” skills than others, or in some situations the stigma attribute is more visible and powerful than in other situations. Stigma can on occasions be “expansive,” pervading all corners of an individual’s biography and identity, and on other occasions “containable,” limited and controllable in terms of consequences and, more importantly, personal and social identity (1).

Hyland (9) in her socio-cultural study of leprosy in Nepal also developed a kind of stigma trajectory in which the different coping styles of the stigmatized people are explained. She described this process as the “concealment cycle.” The difference with the stigma trajectory can be seen immediately in its name. Hyland defines it as a cycle, as a recurrent process and not as a linear process. This is explained below. The concealment cycle is based on the assumption that persons affected will try to conceal their disease for as long as possible, and this is in order to keep their social integrity intact. Social integrity is defined as a person’s place or position in the community. According to the concealment cycle people affected by leprosy will try to manage stigma by concealing the disease and if questions are asked about symptoms, or the person needs to go to the clinic, attention is distracted by telling “stories.” This “story telling” is a kind of concealment in which the person is “saying something and not saying what (it is) wished to keep secret” (emphasis in original) (9). In the next step of
the concealment cycle, the person affected feels that his or her social integrity is threatened. This may happen when questioning or the curiosity of the community increases. To preserve his or her social integrity the person affected may cope by withdrawing. Withdrawal during this stage results in people stopping their treatment, leaving the village together with their families, or leaving alone in order to work in another community.

Hyland (9) then discusses how the stigma can be exposed at different levels: exposure can differ from private exposure to public exposure. Private exposure refers to the person affected knowing that others are talking about him or her. This talking about him or her can change from private curiosity, via suspicion and gossip to “public silence and private slander.” Hyland draws this last term from Miller (10) and it refers to others knowing about it, but avoiding open conflict. Miller (10) argues that people in rural communities in Nepal try to avoid conflict, and in order to provide each other with some “temporal space,” mutual concealment can be chosen. Miller found that it can then happen that in the homes of others, people talk about the disease of the affected person (private slander), but in public people still show politeness and respect (public silence). Others in the community often “wait and see” for the unfolding of events. The study presented in this article demonstrated that this stage of mutual concealment in a social interaction is very useful in understanding that actual stigmatization only occurs after the action (or response) is “triggered” (8). If, during this “wait and see” phase, nothing further happens or when others know the person takes treatment, the process can reverse itself and social integrity is regained (8, 9). If however, the symptoms remain or become worse, or if the person discontinues his or her treatment, the disease can become publicly labelled and result in public discrimination. It is here that the differences between the (linear) process of the stigma trajectory and concealment cycle become clear. In the concealment cycle, a person can regain his or her social integrity, a person can move from a later phase to an earlier phase. In the stigma trajectory such reversal is not possible.

Hyland shows that in the last stage of the cycle, public reactions can differ; from asking the person affected to sleep and eat separately within the family, to living in a separate shelter in the village, or to being sent away. People who were asked to eat and sleep separately or to live separately in the village can regain their social integrity. However, Hyland is not clear in why these reactions differ, the results of the study presented below (8) show that the social differentiation existing in Nepal affected the reactions of others towards people with leprosy. With these findings I elaborated the concealment cycle.

Alonzo and Reynolds (1), and Hyland (9) show that the stigmatization process is a dynamic process, which is continuously shaped and re-shaped, and very much depends on social interaction. Both recognize the importance of the progress of the disease on the impact the stigma has on social interaction. Both studies of Alonzo and Reynolds, and Hyland are very useful in understanding the stigmatization process that occurs and the different phases a person affected by leprosy goes through. The study of Hyland was conducted in western Nepal and the patients interviewed were people who were registered in a specialized leprosy hospital and who already had deformities of the hands and feet. This paper is an attempt to further develop the understanding of the stigmatization process drawing on a study based on interviews with people affected by leprosy who were registered at general health posts. The people interviewed had mostly less advanced leprosy than the people interviewed in the study of Hyland. I did not set out to study the stigmatization process per se; rather this paper reflects themes that emerged from a larger study of understanding people’s adherence to leprosy treatment.

METHODS

The data presented here were drawn from a larger research project on adherence to leprosy treatment from the view of the persons affected by leprosy (8). To accomplish this 76 people were interviewed in depth about their life experiences with leprosy and its treatment. People who had their patient cards in the general governmental health posts situated in the project area of
the Eastern Leprosy Control project (ELCP) were included in this study. Two groups of people were interviewed: people who had completed their leprosy treatment and people who had discontinued their treatment. People were selected based on a random sampling technique. An interview guide was used consisting of 11 themes. People were interviewed in their homes. Interviews lasted for 1½ to 2 hr and were tape-recorded. The recordings were translated and transcribed and were analysed by using a grounded theory approach (13) and the pattern matching methodology as described by Yin (17).

In total, 29 people who discontinued treatment and 47 people who were released from treatment were interviewed. These people lived in different parts of the ELCP area. A good distribution existed between those who lived in rural and urban areas, and those belonging to the different tribes and religions in the area. More men than women were interviewed. More detail about the method and sample is available elsewhere (8).

**RESULTS**

The results of the study illustrate the difference between expected stigma, and experienced stigma. The majority of the people interviewed expected that once others knew about their disease, they would be separated. These expectations resulted in people employing various coping strategies to prevent stigma. However, people whose disease was exposed tried to make their disease less obtrusive by employing other coping strategies. First, I will discuss the strategies employed in managing expected stigma, the so-called strategies of concealment. Subsequently, the coping mechanisms in response to experienced stigma are discussed. These strategies are summarized in Figure 1, which is my development of Hyland’s concealment cycle. Within these discussions the importance of triggers to exposure and discrimination, and the influence of social differentiation on the impact of stigma is shown.

**Strategies of concealment caused by expected stigma.** When asked about their fear and actual actions taken towards them, most of the respondents produced a narrative about other persons who had leprosy and who were treated badly by their communities. These narratives showed something of the expectations the interviewees had of how family and community members would treat them. These narratives were about people being stigmatized and had been told to interviewees by family or community members, or were based on observations they had made themselves. A man who had many patches became very worried after the health worker diagnosed his disease as *Kustha Rog* (Nepali word for leprosy). He remembers images of persons affected by leprosy who were begging in the streets of Calcutta. He said:

I had seen beggars with *Kustha Rog* when I was in Calcutta. When I got *Kustha Rog* myself I used to feel scared thinking about those other people who had *Kustha Rog*... In our village there is a saying that if one of the villagers gets this disease and if another person is close with the infected person, or sits with, eats the *jutho* (a person’s food leftovers) of, or if the husband, or the wife has the disease, then the other person will be in-
affected with the disease. . . In my village the people do not tell me not to walk with them, sit or eat with them, they did not say or do anything. My disease is not clear and that is why most of them could not find out.

The narratives told about other people affected by leprosy however, were always about people who had wounds and deformities and many expected that the development of wounds and deformities would consequently result in being discriminated.

Another important finding related to expectations was that the majority of the people interviewed, including those without wounds, expected that others, after hearing which disease they had, would immediately act negatively towards them. The diagnosis of leprosy was seen as a trigger to discrimination.

Because of these expectations, felt stigma led to a strategy of concealment, which had the effect of reducing the incidence of enacted stigma and retaining one’s social integrity. Almost all interviewees opted for concealment from other community members; 43 (out of the 76) interviewees were able to conceal their disease from their communities. Thirteen people had also concealed their disease from their family members.

In trying to conceal their disease, people employed several strategies to avoid attention. These strategies were triggered by events happening to them. When others asked them questions about symptoms or side-effects they had noticed, or about the interviewed person going to the clinic regularly, many interviewees mentioned that they told “stories.” Stories told were that they had ringworm, had a simple skin disease, had become black because of the sun, had a wound because of the type of work, or needed treatment because of another disease. The function of these “stories” was to distract attention.

If the symptoms of leprosy developed or increased, the questioning and curiosity of other people about their symptoms increased, and the respondents perceived that their social integrity was threatened. In this stage they tried to manage the stigma by avoiding situations in which enacted stigma could be triggered, or to diminish the number of triggers. Some people, when they had visible signs of leprosy, withdrew temporarily until their symptoms had improved. One woman, who was a widow, developed some white patches on her hands and face. After people questioned her about it, she took her children with her

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**Fig. 1.** The stigmatization process.
and went to a family member in a large city nearby. There, she started treatment and remained in the city until the patches were less visible. Another man, who developed a patch on his face which became swollen and festered at the beginning of his disease, reported how he tried to stay inside the house or around the house until the swelling went. Some went early to their fields to avoid meeting other community members.

The concealment strategies employed were very much linked to the hierarchical position of the individual within their community and their family. To some the impact the stigma had on their lives was less than on others. Family and community status influenced the reaction of others towards them.

The majority of the people with a higher status succeeded in concealing their disease from other community members. Normally, when a person developed many patches, questions were asked, however people with a higher status believed they were protected against questions being asked. (Note: Interviewees mentioned several factors to differentiate themselves from others and which they believed showed their status within the family and community. I used these factors to determine a person's status within the family and community. These factors and their consequent classification are given in the appendix.) Questions were asked of some people with a higher status, but they could avoid the questions by telling a "story." A few people stated that they believed that others were gossiping about them. Half of the people with a lower community status were able to conceal their disease, but questions were asked of relatively more people with a lower community status. Their status in the community did not protect them from these types of questions. Some mentioned that they had to accept the behaviour of others. They said things like:

How could I discuss it with them. Of course, they scolded me. Whose mouth can I keep shut? ... Then we understand ourselves and keep quiet.

Another example gives the comments of the wife of a person affected by leprosy on the negative behavior of villagers:

Politicians should make some arrangements for the poor people, but they do not care about us. There would be no difference between rich and poor people if they took a little care of the poor people. We are poor people and that is why other people come here and beat my husband but the villagers do not come and stop them.

Miller (10) in his discussion on decision-making in villages in Nepal, argued that it is not so important what people think about each other, but what people say about each other; it is words that give weight to attitudes. Although people with a higher status who had visible signs of leprosy may still fear the exposure of their disease, their status in the community protected them from others asking questions, and thus from actions being taken.

The results of the analysis showed that within the family different hierarchies could be identified, based on decision-making power, gender, and age and within these between the people who could contribute to the family income and those who could not (see appendix). Men with decision-making power who could contribute to the family income all concealed their disease from other family members. Some had made their decision not to inform other family members as an attempt to avoid causing family concern. One man said about this:

I did not tell my family about the disease. I was afraid they would be tense and get worried. We do not have enough food to eat and clothes to wear. I thought that whatever happens, will happen to me only.

These people could conceal their disease because, due to their position, other family members could not question them about their disease. As one man said, "I am the guardian of the house and I can decide what to do." Such an attitude makes it difficult for others to inquire about a disease which they might believe to be dangerous. People who did not belong to this group of decision makers were very much aware of their position within the family and they expressed it as vital that they contributed to the family income. These people lived dependent lives, and were greatly influenced...
by other family members, and thus contributing to the family income provided them with a certain status or secured their position within the family. The felt stigma among them was very high.

**Strategies employed in managing experienced stigma.** During the interviews, it was not easy for the interviewees to discuss the actual stigmatizing behavior of family and community members towards their disease. Many tried to avoid the subject and many cried when they described the behavior experienced. Thirty people mentioned that their disease was known to their communities, of whom 20 experienced negative behavior from other community members. Six people mentioned that their community members were “waiting to see” what would happen, and only four did not experience any negative behavior from others. In cases where the other family members knew of their disease, negative family behavior was less common. This happened to 10 of the 60 people. The majority of the interviewees received family support.

After their disease was exposed to other people the respondents were not immediately publicly discriminated against. In this phase, people whose disease was exposed to other community members adopted a strategy of “covering;” they took all possible steps to reduce the salience of their stigma for others. They were living in constant fear that they would be sent away from their families and be expelled from the village. Some people withdrew from social life, by going to their fields in the early morning, by working outside the village, or by staying near the house. Two people started working as migrant laborers in India and only came back after their symptoms had diminished. Others stayed in the village, but avoided confrontation by sitting apart, not sharing utensils, not visiting public gatherings, etc. Some people tried to stay at home and work near the house. They tried to live and work unnoticed and had developed a submissive attitude.

As I argued above, in rural communities in Nepal people try to avoid conflict and provide each other with some temporal space. As this period of mutual concealment could last for a long time I elaborated the concealment cycle with a separated phase, the phase of mutual concealment. In this phase, people may gossip behind their backs and talk about the disease of the affected person (private exposure), but in public people still show politeness and respect. People “wait and see” what will happen.

The results of the analysis show that actual public discrimination only occurred in response to one or more triggers. Several negative triggers existed that led to exposure of the disease and people tried to diminish the effects of these triggers and situations in which these triggers would be obtrusive. Negative triggers that resulted in public exposure were increase in visible symptoms, development of wet wounds, the regular visits to the clinic, specific side effects, like reddish-brown discoloration of the skin, swelling and weakness, and inability to contribute to the family income. A relationship was found between status and the type of negative actions taken. For people with a higher community status, only persistent and severe wounds were triggers to other community members acting negatively towards them. For this reason, some people with a higher status were no longer visited by other community members. However, these negative actions could be temporary and were situation specific. In this group of people with a higher status, no one was threatened with expulsion from the village. There were more negative actions directed against people with a lower status and after they had developed a severe and persistent wound, they experienced more severe negative actions Some people were even threatened with expulsion or had been already expelled. The majority of the people whose disease was exposed and who experienced negative behavior from others, believed they were not in a position to do anything about it and had to accept what was happening, or as one interviewee said:

The villagers, my neighbors know about my disease. Sometimes when I get into an argument people say bad things to me, like “you have Soon Bairi” (leprosy). At that moment I feel really bad, and keep quiet thinking ‘what to do if I do have that disease.

Of the people whose disease had been exposed to their families, this knowledge was in some cases only shared by their closest
family members (wife, husband, or parents), while in other cases it was only known to the nuclear but not the extended family. Some had informed their family members themselves of the diagnosis of leprosy, while others had delayed this and it had been exposed later, after other family members had found the medicines, questioned the regular visits to the health post, or the interviewee had made an admission after a long period of being questioned about it. Due to the fact that leprosy was viewed as being very contagious, many interviewees had adapted their behavior, or had been asked to change it to avoid infecting other family members. Most of them did not give their jutho to other family members and stored their food utensils separately, and in some cases even their bedding and clothes. Some interviewees withdrew themselves from certain aspects of family life; they started to sit, eat, and sleep separately, but continued to join the family in other activities. A few were no longer touched by others, or were asked to live separately on the family compound, outside the family houses. For most, this separation was only temporary. After the symptoms had diminished or the wounds had healed, the person would be allowed to move back into the house. One man developed a wound, and only after the advice of other people did he go to the health post. Here they diagnosed his disease as Kustha Rog. He informed his family about the diagnosis and was then asked to eat and sleep separately until his wounds healed and he moved back into the house. He said:

No one in my family ate with me for at least two to three months, because they thought this disease was contagious. All of them felt bad and advised me to take my medicines continuously. They said that taking the medicines could cure my disease.

The different family hierarchies had an impact of the effects of stigma and coping strategies employed. Decision-makers withdrew themselves from family life to avoid transmission and of fear of the social consequences. The position of non-decision-makers within the family did not change much as long as they showed submissive behavior and contributed to the family income and as long as they did not develop severe wounds, weakness, or illness. Some differences were found in comparing women with men who were not decision-makers. Visible signs of leprosy did not result in negative actions against men who were not decision-makers. However, visible signs of leprosy were triggers for negative actions towards some women. The women who had not experienced negative family behavior belonged to better-educated families, or where knowledge about leprosy and its treatment existed. Further, age was also an influencing factor; in the group of people above 50 yrs, it was found that to these people it was important to contribute to the family income. They helped their families by doing minor work. After developing wounds and deformities with which they could not work anymore, four people were sent out of their villages. Interviewees who were less than 15 yrs old had all received a great deal of family support. These children were kept at home.

**DISCUSSION**

The stigmatization process as an interactive and dynamic process. The results of the analysis demonstrate that a stereotypical view about leprosy is still dominant. Although the majority of the interviewees expected to be separated, few people were actually expelled from the village or separated within the community or family. In the past when no treatment existed for leprosy, people were sent out of the villages due to fear of transmission, and thus the stereotypical thinking is mostly based on this past reality. This study shows that people employ a variety of coping strategies to manage the stigma attached to leprosy. As the stigmatization process is based on the interaction between people, the strategies employed by individuals depend very much on the expected and experienced (re)actions of others towards them. The different phases of this stigmatization process are demonstrated in Figure 1, and described above. This stigmatization process is an elaboration of the concealment cycle as developed by Hyland (7). The original concealment cycle is extended with the phase of mutual concealment. After the disease is exposed to others this does not immediately result in public discrimination. A kind of “wait and
The importance of triggers. The results of the analysis showed that going to a subsequent phase in this stigmatization process is always triggered. The type of triggers had an impact on the coping strategies and also on the type of stigmatizing actions of others. Not only visible symptoms of the disease were perceived as triggers to exposure and discrimination, also other events related to the disease and its treatment were perceived as triggers. Other triggers to exposure and discrimination included regular visits to the clinic, the blisterpack of the medicines, and the side-effects of the Multi-drug Therapy treatment (esp. weakness, and a darkening of skin due to the clofazimine). Next to these so-called negative triggers, some positive triggers were also reported. The knowledge that the interviewees were on treatment resulted in others being willing to wait and see what would happen. Likewise, improvements observed in the symptoms also contributed to a “wait and see” behavior pattern. Thus, depending on the type of trigger, a positive or a negative trigger, stigmatization can become more severe or diminish. In the elaborated cycle a person can move to a subsequent phase into the model or can move back to a previous phase.

The importance of social differentiation in stigma. Managing stigma has to be regarded in a wider context. The impact of stigma is related to individuals’ position in the family and community hierarchies. Social interaction between people is based on social rules and norms that are related to their position within the hierarchies. As stigmatization is a dynamic process and is based on interactions between people. The impact stigma has on a person’s life reflects the general way people interact in the wider society. Because of their authority within the community, high status people could not be asked “curious,” personal questions. Even after they had developed a stigmatizing disease, other people could still not ask them questions, and negative behavior was only shown if the disease reached an advanced stage. Lower status people were already vulnerable to scolding in their everyday life and were never permitted to attract attention. Where, in other circumstances, any mistake or failure would be noticed and questioned, here the disease leprosy was the trigger for negative actions. Leprosy legitimized negative actions. This study demonstrates that the stigma in Nepal could not only exist, as Waxler (15) stated, because of the hierarchical nature of Hindu society, but that the hierarchical society also explains the differences in the impact of stigma between people holding different positions within the hierarchies, or as concluded by Parker and Aggleton (11) stigma feeds upon, strengthens and reproduces existing inequalities of class, race, gender and sexuality.

CONCLUSION

What are the implications of these findings for interventions aimed at reducing the stigma of leprosy? As leprosy is still related to images of people with deformed hands and/or feet and who are sent away. It is not surprising that the majority of the patients, when asked about strategies for stigma coping, recommend to keep the diagnosis leprosy a secret or even to avoid contact with other people. Communities need to be targeted with information regarding leprosy and stigma, efforts should be made to change negative attitudes and practices. Particularly the linkage with the stereotypes dangerousness and infectiousness (5-8) provokes adverse reaction among the public. Therefore, messages like “leprosy is not infectious anymore after starting treatment” should be one of the key targets for anti-stigma interventions. Research done within the mental health field showed that strategies aiming at education and contact had impact on the attitudes about mental illness (4). Members of the general public who interacted with a person with mental illness exhibited large changes in stigmatizing attitudes. Further, interventions are needed on the individual level, aiming at empowering the affected individual. This could include counselling, learning coping skills and self management, meeting places, practical assistance, and peer support.

Acknowledgment. I am indebted to both Ann Taket and Rayah Feldman (both of the South Bank
Appendix. Different factors determining a person’s status.

- **Land ownership** and size of the land. This was about what the person affected described concerning income received from the land, and whether its produce was sufficient to live on for a whole year, without the need for supplementary paid work.

- **Occupation.** Type of occupation of all family members. I used the categories developed by Rao (1992)*. He defined nine categories for occupation: landless labor, sharecropper, land owner, employer on land, non-agricultural service, artisan, owner of an industry, other occupations, and unemployed.

- **The educational level** of the patient or other family members. For some affected persons interviewed, it was very important to mention that they could read. The ability to read gave them a certain status or position within the community.

- **Type of family.** Being a nuclear or joint family, and type of family structure. Some joint families were those that had lived in the community for many generations. This and the presence of several married brothers gave the family a higher status.

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   http://www.cia.gov
The type of housing the family had. It made a difference if the family lived in a wooden, two-story house or in a hut made of mud or straw.

Political status. Some people stated that they were active members of a political party. Being an active member of a political party means that people can influence some decisions regarding the whole village, such as planning of roads, taxes, etc.

Ritual status. Some people were traditional healers, or were Brahmin priests, which gave them a higher status.

Different cross-cutting factors acted together to give rise to a person’s status. If for example, a person was a landowner, I categorized this person as being of higher status. If a person lived in a mud hut, was a day laborer and mentioned that he or she had days without income and thus food, I categorized this person as having a lower status. Active members of a political party, or people with a ritual status, or shopkeepers, were mostly categorized as higher status, though not all factors were congruent. The different categories as used in this study are shown below.

### Status in the community.

**Higher community status**
- Protected against questions being asked.
- Majority succeeded in concealing their disease from other community members.
- Only persistent and severe wounds were negative triggers resulting in negative behavior of others towards them.
- No expulsion reported.

**Lower community status**
- Had to accept the behavior of others.
- Only half of them were able to conceal their disease from other community members.
- Experienced community pressure.
- Negative actions reported, including expulsion.

### Status in the family.

**Decision-making power**
- Hold a more independent position in the family.
- On occasions, they withdrew from family life to avoid transmission and some concealed their disease out of fear of the social consequences.
- Negative triggers were having severe (wet) wounds and some toes missing.

**No decision-making power**
- Dependent of other family members.
- Their situation did not change much so long as they showed submissive behavior and contributed to the family income and did not develop a severe wound, weakness, or illness.
- Minor symptoms could lead to negative family behavior.

**Men**
- Men constituted the majority of those with decision-making power.
- Men with decision-making power concealed out of concern for the other family members, the other men out of fear of exposure.

**Women**
- Women tried to conceal their disease and showed a submissive attitude.
- Had to accept the behavior of others, and were very vulnerable to negative actions.

**Old age**
- On occasions the oldest person in the family was not respected and sometimes even regarded as a burden. The ability to contribute to the family income was important.

**Child**
- Out of concern about their children’s future all children affected by leprosy received family support.
A Comparison of Economic Aspects of Hospitalization Versus Ambulatory Care in the Management of Neuritis Occurring in Lepra Reaction

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ABSTRACT

Neuritis is one of the important causes of deformities and disabilities in leprosy. Neuritis has been managed both in the field and in hospital. This study was done to compare the economic aspects of cost of ambulatory vs in-patient management of neuritis in leprosy. The quality of life of the affected patients and the clinical improvement in the 2 groups were also studied. Twenty six patients fulfilling the study criteria were randomized into the ambulatory and in-patient group (13 in each group). The primary outcome examined was cost, in various categories; the secondary outcomes included pre- and post- treatment comparison of Quality of Life (QOL) scores and tests of sensory and motor function.

The direct and indirect medical costs incurred by patients in the hospitalized group were higher than those patients in the ambulatory group. The difference in the direct medical costs between the two groups was Rs. 9110.5, and the extra direct non medical costs incurred by patients in the hospitalized group was Rs. 888.50 because of more frequent visits of family members. A greater percentage of ambulatory than in-patients returned to work in ≤15 days (53.8% vs 15.3%), and the mean duration before returning to work was 19.5 days ambulatory patients compared to 66.8 days for in-patients group. The QOL scores and motor and sensory function tests showed no significant difference between groups. Although the sample size was small, these preliminary results suggest that substantial cost minimization by ambulatory care is possible without significantly affecting the quality of life or peripheral nerve function.

RÉSUMÉ

Les névrites sont une des causes importantes de difformités et de handicap de la lèpre, qui peuvent être traitées à la fois sur le terrain et à l’hôpital. Cette étude fut entreprise afin de comparer les aspects économiques du coût des soins ambulatoires versus hospitaliers des névrites lépreuses. La qualité de vie des patients et l’amélioration clinique fut aussi étudiée dans les 2 pratiques. Vingt-six patients remplissant les conditions de l’étude furent assignés au hasard soit au groupe ambulatoire, soit au groupe hospitalisé (13 par groupe). Le paramètre principal étudié fut le coût pour chaque catégorie; les résultats secondaires ont inclus la comparaison avant et après le traitement des scores de qualité de vie (QDV) et des tests de fonction sensorielle et motrice.

Les coûts directs et indirects incombant aux patients hospitalisés furent plus élevés que ceux du groupe ambulatoire. La différence entre les coûts directs de chaque groupe a été de Rs. 9110,5 et les frais directs non médicaux des patients hospitalisés ont été de Rs. 888,50, dû aux visites plus fréquentes des membres de la famille. Un plus grand pourcentage de patients ambulatoires est retourné au travail dans les 15 jours (53,8 versus 15,3%) et la durée moyenne avant de retourner au travail a été de 19,5 jours pour les patients ambulatoires comparée à 66,8 jours pour les patients hospitalisés. Les scores QDV et les tests fonctionnels sensoriels et moteurs n’ont pas révélé de différence significative entre les deux groupes de patients. Bien que la taille de l’échantillon étudié ait été faible, ces résultats préliminaires...
The economic aspects of the management of neuritis in leprosy have received scant attention. A recent paper by Naik and Ganapathy highlighted the need for such studies in the present declining phase of leprosy endemicity (9). Leprosy causes a variety of impairments both primary and secondary. The significant, serious, and more common primary impairments resulting from leprosy are mostly related to the consequences of nerve damage secondary to neuritis. There have been many studies (3, 4, 6, 8, 12) on the effect of steroids on neuritis of leprosy conducted both in the field and in hospitals but none comparing the implications of the cost of the two modes of treatment i.e., hospitalization and ambulatory management. Along with the study on cost, quality of life and motor and sensory functions were also studied. There are only a few studies done on the psychological impact of the disease and quality of life in patients with leprosy (5, 7, 11). The aim of the present study was to compare the economic aspects of ambulatory and in-patient management of neuritis in leprosy. The impact of these two modes of management on the quality of life scores and the motor and sensory functions were also looked at.

MATERIALS AND METHODS

The study was conducted at the skin and leprosy department of a tertiary level teaching hospital in Tamilnadu from October 1999 to March 2001 incorporating a multidisciplinary team of dermatologists, neurologists, and a health economist. All patients presenting with neuritis, as a part of either type 1 or type 2 reactions, of less than 6 months duration, were included. Neuritis was defined as tenderness of the nerve and/or deterioration in sensory or motor function. Patients with a nerve abscess, pustular or ulcerating erythema nodosum leprosum (ENL), and those on oral or parenteral steroids during the month prior to entering the study were excluded. Children below the age of 12 yrs were also excluded.

Patients, who were willing to enter the study after informed consent, were randomized into ambulatory and in-patient (patients who received treatment in hospital) groups. A computerized random numbers table was used for randomization in blocks of two. These patients were classified based on findings of clinical examination and skin smears, using the Ridley-Jopling classification.

Sample size. The sample size was calcu-
lated based on the primary outcome. The assumption was that 5% of the patients being managed in the admitted group would return to work in 15 days, whereas 45% of patients being managed on an ambulatory basis would return to work in 15 days. Two weeks are the period of admission for the in-patient group and stipulated period of rest in the ambulatory arm. With a type 1 error of 5% and type 2 error of 20% the estimated sample size was 18 in each group.

Outcome measures. **Primary outcome**: Day of return to work after the stipulated period of rest/admission. **Secondary outcomes**: (i) Estimation of mean cost/patient; (ii) Improvement in the score of QOL; (iii) Improvement in sensory and motor scores.

**Protocol for management.** Patients in the in-patient group were admitted for 2 weeks and were monitored in the ward for complications of steroid therapy. Those in the ambulatory group were educated regarding the complications of steroids and advised rest at home 2 weeks. Patients in both groups were given prednisolone at 1 mg/kg/day. Follow-up was done every 2 weeks during the first month and monthly thereafter, until the end of steroid treatment. The dose of steroids was reduced by 10 mg every visit. The day of return to work was recorded. A detailed clinical examination, sensory and motor assessment was done as a baseline, at the end of 2 weeks and then monthly till the end of treatment.

All patients underwent a pre-steroid work up which included hemoglobin, complete blood count, urine routine, random blood sugar, and a chest X-ray to rule out tuberculosis.

**Sensory assessment.** Sensory examination was done using Semmes-Weinstein graded nylon filaments. Palms were tested with filaments of 0.2 gm (blue) and 2 gm (purple), and the soles with 4 gm (red) and 10 gm (orange).

Protective sensation was detected by using purple filaments for the palms and orange for the soles. Sensation was tested on 10 standard points on the palms and soles. One point was given to each area with mis-referred or absent sensation. The maximum score for each ulnar nerve was 4, median nerve was 6 and posterior tibial was 10. Each site with absent sensation was scored and the mean sensory score for each nerve before and after treatment was calculated in both groups.

**Motor power.** Motor power of the muscles of hands and feet were tested and graded according to the Medical Research Council scale on a score of 0 to 5. The mean motor score prior to and after treatment was calculated.

**Cost analysis.** A detailed proforma was filled with regard to direct medical costs, direct non-medical costs and indirect costs (Annexure 1) (All Annexures for this article are available in the online issue of the Journal at www.leprosy-ila.org). Direct costs are the costs incurred by the health sector and the patient. The direct medical costs include cost of medications, tests and hospitalization. The direct non-medical costs include cost of transport and food. Indirect costs include the wages lost on account of illness (1, 2). The details of unit cost of each item used for the estimation of the direct medical costs is given in Annexure 1.

**Quality of life assessment (QOL).** Quality of life is defined as an individual’s perception of their position in life in the context of the culture and the value systems where they live, and in relation to their goals, expectation, standards and concerns (13).

The QOL questionnaire was filled out for patients in both groups before starting steroids and at the end of treatment. The questionnaire adopted for this study was modified from the WHO Quality of life Global pool of questions (Annexure 2) (see online Journal). These include information on 5 domains-physical, psychological, level of independence, environment, and social (Table 1). Each question had a maximum score of 5 except the question on pain in the physical domain, which had a maximum score of 6. The total maximum score was 106. A higher score meant a better quality of life. The total and l mean scores of each of the domains were calculated and the improvement in the score was compared among the 2 groups. The mean score for the sub-groups with and without deformities was calculated and compared.

**Data analysis.** Intention to treat analysis was done using the EPI INFO package. Since sample size was small and not normally distributed non parametric tests (Kruskhal-Wallis H test) were done to determine the statistical significance of ob-
served differences in costs, QOL scores and motor and sensory scores in the 2 groups. Chi-square test was done to test the difference in proportion of patients who returned to work.

**RESULTS**

**Demographic and clinical data.** Five hundred and eleven leprosy patients were examined during the study period. Of them, 53 (10.37%) were diagnosed as having reactions. Twenty-six patients were found eligible and willing for the study and were randomized into the ambulatory and in-patient group (13 in each). The baseline characteristics of the two groups were comparable with respect to age, sex, presenting complaints, reaction type, deformity and the spectrum of leprosy. (Table 2) Twenty-three nerves in 13 patients of the ambulatory group and 28 nerves in 13 patients in the in-patient group were involved. The ulnar nerve was the most common nerve involved in both groups. There were 4 defaulters: two from the ambulatory group and one from the in-patient group were lost to follow-up after the first visit, and one other patient from the in-patient group was lost to follow-up after 3 months.

**Cost analysis.** (Table 3) Considering return to work as the outcome, patients in the ambulatory group, on an average, returned to work is 19.5 days (0 to 60 days, median = 13) as compared to 66.8 days (0 to 180 days, median = 47) taken by patients in the in-patient group. This difference was statistically significant (p = 0.02).

The proportion of patients who returned to work in ≤15 days in the ambulatory group was 7 (53.8%) as compared to 2 (15.3%) in the in-patient group. The difference was statistically significant (p = 0.04).

The mean direct medical cost in the ambulatory group was Rs.2341.30 and of the in-patient group was Rs.11451.80. This difference of Rs.9110.50 was statistically significant (p = 0.001). This extra cost incurred for the hospitalized patients is mainly because of bed, investigations, diet and professional charges of the doctor and the nurse. The average direct non-medical costs were Rs.348.30 and Rs.1236.80, respectively in the ambulatory and in-patient group. The extra cost of Rs.888.50 incurred by the patients in the in-patient group was mainly because of more frequent visits of the family members. The total extra cost to the hospitalized patient incorporating both medical and non medical cost was on an average Rs.9999.00.

The indirect cost was estimated on an average, as Rs.4544.30 and as Rs.13051.10, respectively for the ambulatory and in-patient group. The difference of Rs.8506.80 is the economic gain in the ambulatory group.

Total cost for a patient including direct and indirect cost was Rs.7233.90 and Rs.25,739.70, respectively for the 2 groups (ambulatory and in-patient). The total extra direct and indirect cost per patient in the hospitalized group was Rs.18505.80. The overall difference between the ambulatory and inpatient groups ranged from Rs.2655–34,356 based on the estimates of 95% CI for the mean cost of ambulatory care together with inpatient care. This is the money lost per case if we have a policy of
hospitalization for all cases of mild to moderately severe neuritis.

**Analysis of quality of life in the 2 groups.** Seventeen patients, 9 in the ambulatory group and 8 in the in-patient group were available for assessment of pre- and post-treatment QOL scores.

The sum and mean of pre- and post-treatment scores of all the domains in both groups are shown in Table 4 and 5, respectively. The mean pre-treatment scores in the ambulatory and inpatient group were 61.3 and 68.0, respectively. The mean post-treatment scores in both groups were 77.7 and 78.8, respectively. The overall mean difference in pre and post treatment scores for ambulatory and inpatient groups was 10.8 (95% CI 6.7 to 14.4) and 16.4 (95% CI 12.2 to 20.6), respectively. The difference was not statistically significant (p = 0.42).

Patients with and without deformity were compared. The mean score before treatment was 65 in patients with ulnar claw hand and 62.3 without it. The score for patients with

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**Table 2.** Shows the baseline characteristics of patients in the ambulatory and inpatient group.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ambulatory (N = 13)</th>
<th>Inpatient (N = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (Range)</td>
<td>31.3 (15 to 49)</td>
<td>40.7 (19 to 60)</td>
</tr>
<tr>
<td>Sex: Male:Female</td>
<td>11:2</td>
<td>12:1</td>
</tr>
<tr>
<td>Presenting symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin lesions</td>
<td>7 (53.8)</td>
<td>7 (53.8)</td>
</tr>
<tr>
<td>Neural</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>3 (23.07)</td>
<td>3 (23.07)</td>
</tr>
<tr>
<td>Sensory impairment</td>
<td>1 (7.6)</td>
<td>2 (15.3)</td>
</tr>
<tr>
<td>Motor deficit</td>
<td>5 (38.4)</td>
<td>6 (46.1)</td>
</tr>
<tr>
<td>A combination of the above 3 symptoms</td>
<td>4 (30.7)</td>
<td>2 (15.3)</td>
</tr>
<tr>
<td>Reaction in relation to period of treatment with MDT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 6 months</td>
<td>6 (46.1)</td>
<td>9 (69.2)</td>
</tr>
<tr>
<td>6 months</td>
<td>5 (38.4)</td>
<td>3 (23.07)</td>
</tr>
<tr>
<td>Post MDT</td>
<td>2 (15.3)</td>
<td>1 (7.6)</td>
</tr>
<tr>
<td>Recurrence of reaction</td>
<td>4 (30.7)</td>
<td>4 (30.7)</td>
</tr>
<tr>
<td>Reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>12 (92.3)</td>
<td>12 (92.3)</td>
</tr>
<tr>
<td>Type 2</td>
<td>1 (7.6)</td>
<td>1 (7.6)</td>
</tr>
<tr>
<td>Neuritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulnar</td>
<td>11 (47.8)</td>
<td>11 (37.9)</td>
</tr>
<tr>
<td>Median</td>
<td>3 (13.04)</td>
<td>5 (17.2)</td>
</tr>
<tr>
<td>Common peroneal</td>
<td>4 (17.3)</td>
<td>8 (27.5)</td>
</tr>
<tr>
<td>Posterior tibial</td>
<td>5 (21.7)</td>
<td>3 (10.3)</td>
</tr>
<tr>
<td>Facial</td>
<td>0</td>
<td>2 (6.8)</td>
</tr>
<tr>
<td>Deformity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>3 (23.07)</td>
<td>1 (7.6)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>6 (46.1)</td>
<td>5 (38.4)</td>
</tr>
<tr>
<td>Grade II</td>
<td>4 (30.7)</td>
<td>7 (53.8)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT</td>
<td>6 (46.1)</td>
<td>6 (46.1)</td>
</tr>
<tr>
<td>BL</td>
<td>3 (23.07)</td>
<td>4 (30.7)</td>
</tr>
<tr>
<td>LL</td>
<td>1 (7.6)</td>
<td>1 (7.6)</td>
</tr>
<tr>
<td>Subpolar LL</td>
<td>0</td>
<td>1 (7.6)</td>
</tr>
<tr>
<td>Pure neuritis</td>
<td>3 (23.07)</td>
<td>1 (7.6)</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>high</td>
<td>8.3%</td>
<td>8.3%</td>
</tr>
<tr>
<td>low</td>
<td>91.7%</td>
<td>91.7%</td>
</tr>
</tbody>
</table>
and without foot-drop were 61.2 and 63.25. None of the differences reached statistical significance.

Assessment of sensory function in the 2 groups of the study. The mean sensory scores of the ulnar, median and posterior tibial nerves improved with treatment in both groups. The difference in improvement seen was however not statistically significant (Table 6).

Assessment of motor function in the 2 groups of the study. The mean motor scores of the ulnar nerve of the patients in the ambulatory group and ulnar, median and common peroneal nerves of patients in the in-patient group improved with treatment (Table 7). Only the difference in improvement of the median nerve scores in the in-patient group reached statistical significance (p = 0.01). There was deterioration of the mean motor scores of the median and common peroneal nerves of patients in the ambulatory group as compared to the in-patient group, but this was not statistically significant.

DISCUSSION

This is a preliminary study on the comparative economic analysis of the treatment of neuritis in hospitalized and ambulatory patients. The results of this study need to be viewed with caution in view of the small sample size which is a major drawback. However, the study does provide some interesting data which are discussed below since there is paucity of studies relating to the economic aspects of treatment policies for neuritis. Return to work was considered

TABLE 3. Shows the different categories of costs in rupees in the two groups.

<table>
<thead>
<tr>
<th>Categories of cost</th>
<th>Ambulatory</th>
<th>Inpatient</th>
<th>p value</th>
<th>Extra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct medical cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2,341.30</td>
<td>11,451.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1,526</td>
<td>9,086.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(566.65 to 11,546.55)</td>
<td>(6,505.25 to 27,128.69)</td>
<td>0.001</td>
<td>9,110.5</td>
</tr>
<tr>
<td>Direct non-medical cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>348.30</td>
<td>1,236.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>287</td>
<td>705</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(45.4 to 960)</td>
<td>(49 to 6500)</td>
<td>0.07</td>
<td>888.5</td>
</tr>
<tr>
<td>Indirect cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4,544.3</td>
<td>13,051.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>3,900</td>
<td>4,695</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(560 to 11,200)</td>
<td>(1,800 to 72,100)</td>
<td>0.12</td>
<td>8,506.8</td>
</tr>
<tr>
<td>Total cost</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7,233.90</td>
<td>25,739.70</td>
<td></td>
<td>18,505.80</td>
</tr>
<tr>
<td>Median</td>
<td>3,702.5</td>
<td>10,905.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2,638.2</td>
<td>6,293.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>(3,638.2 to 10,829.5)</td>
<td>(6,293 to 45,186)</td>
<td>&lt;0.01</td>
<td>(2,655–34,356)</td>
</tr>
</tbody>
</table>

TABLE 4. Shows the distribution of sum of quality of life scores according to domains among patients in both groups.

<table>
<thead>
<tr>
<th>Domain</th>
<th>Ambulatory (N = 9)</th>
<th>Inpatients (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-treatment</td>
<td>Post-treatment</td>
</tr>
<tr>
<td>Physical</td>
<td>127</td>
<td>184</td>
</tr>
<tr>
<td>Independence</td>
<td>138</td>
<td>159</td>
</tr>
<tr>
<td>Environment</td>
<td>143</td>
<td>145</td>
</tr>
<tr>
<td>Psychological</td>
<td>96</td>
<td>143</td>
</tr>
<tr>
<td>Social</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>531</td>
<td>663</td>
</tr>
</tbody>
</table>
to be a sound surrogate marker for effectiveness of treatment and well being of the patient. A statistically significant number of patients in the ambulatory group returned to work early (p = 0.04). The time taken to return to work was also significantly shorter among patients in the ambulatory group. This implies a significant gain in productivity.

The patients in the in-patient group returned to work later. Some patients continued to stay away from work after discharge from hospital. It is possible that hospitalization, which has a known negative effect on the patient’s view of the disease, influenced this outcome (11). The direct medical costs were significantly higher in the hospitalized group. The difference in costs were due to the bed and nursing charges, doctors’ professional fees and diet in the hospitalized group. The mean overall cost for ambulatory group was Rs.7,233.9 (95% CI 3638.2 to 10829.5) and for hospitalized group Rs.25739.7 (95% CI 6293 to 45186). The economic gain reflected by the differences of these two costs was Rs.18505.8. The lower and upper estimates of this mean cost difference was calculated from the difference of the lower and upper limits of the 95% CI. This ranged from Rs.2655 to 34,356. This reflects the range of economic gain possible if the policy of ambulatory care of patients with neuritis is adopted. The high cost of hospitalization and the loss in productivity following this, were the main disadvantages experienced by opting for this mode of management for neuritis. Thus the results of our study suggests that it is economically more advantageous to adopt the ambulatory management of neuritis, especially in resource poor countries.

In a study published earlier on the impact of leprosy on the QOL, it was seen that the mean score of QOL was lower in cases than controls in all domains except spiritual (94.5 for cases Vs 101.5 for controls). Males with visible deformities had a significantly lower score than those without deformity (91.4 vs. 99.2). There was a positive correlation between the socio-economic status and quality of life scores (9). In our study physical, psychological and levels of independence showed an improvement. However, there was no difference in the pretreatment mean QOL among patients with and without physical deformities (63.1 vs. 62.5). One of the reasons for the apparent lack of impact of deformity in the QOL score could be the small sample size stud-

<table>
<thead>
<tr>
<th>Table 5. Shows the results of pre- and post-treatment quality of life scores in both groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>Pretreatment Mean</td>
</tr>
<tr>
<td>S.D.</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>Post-treatment Mean</td>
</tr>
<tr>
<td>S.D.</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>Difference</td>
</tr>
<tr>
<td>95% CI</td>
</tr>
</tbody>
</table>

*p = 0.42.

<table>
<thead>
<tr>
<th>Table 6. Shows the mean and total sensory scores of patients in both groups pre- and post-treatment.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerves</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Ulnar</td>
</tr>
<tr>
<td>*0.2 g</td>
</tr>
<tr>
<td>*2 g</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>*0.2 g</td>
</tr>
<tr>
<td>*2 g</td>
</tr>
<tr>
<td>Post tibial</td>
</tr>
<tr>
<td>*4 g</td>
</tr>
<tr>
<td>*10 g</td>
</tr>
</tbody>
</table>
ied. It is also possible that the questionnaire was not sensitive enough to detect the impact of deformities on the QOL in leprosy patients. It has been reported that patients with grade 1 disability face less discrimination in the family and at work compared to those with grade 2 disabilities (10). In our study, 58% of patients had either no deformity or grade 1 deformity. In another study done in Tamilnadu it was seen that caste status influenced the nature and the extent of handicaps experienced by leprosy patients (7). This in turn could have influenced the QOL. Thus the impact of deformities on the patient’s well being and attitude to the disease is multi-factorial and was probably not adequately addressed by the questionnaire used. However, since there is no significant difference in the quality of life between the ambulatory and hospitalized group it may be concluded that status of hospitalization did not contribute significantly to improvement in the QOL.

In conclusion, it may be said that the burden of disease in leprosy has not received adequate attention. Large multicenter studies are required to address the economic and other non medical aspects of the management of the leprosy.

Acknowledgment. The help given by Mrs. Linda Roberts, Secretary and Mr. Jamaluddin is gratefully acknowledged.

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Susceptibility to Leprosy May Be Conditioned by an Interaction between the NRAMP1 Promoter Polymorphisms and the Lepromin Response

Frederico Rogério Ferreira, Luiz Ricardo Goulart, Heyder Diniz Silva, and Isabela Maria Bernardes Goulart

ABSTRACT

Controversial results have been achieved by attempting to associate the NRAMP1 gene with Mycobacterium leprae susceptibility as well as with the Mitsuda reaction, which represents a specific immune response to M. leprae. This study evaluated this association as well as the interaction of the polymorphism (GT)_n in the promoter region of the NRAMP1 gene with a specific immune response to M. leprae measured by the intradermal Mitsuda test in leprosy patients and in non-consanguineous household contacts. The study aimed to evaluate the association of this gene polymorphism with resistance or susceptibility to the disease, and/or with clinical forms of the disease, in a population in an endemic area served by the State Reference Center in Sanitary Dermatology and Leprosy, Federal University of Uberlândia, MG, Brazil. Leprosy patients (90) were diagnosed according to Ridley and Jopling criteria and they grouped into multibacillary (MB) and paucibacillary (PB) patients. The control group consisted of 61 non-consanguineous contacts. NRAMP1 promoter genotypes were obtained through amplification by the polymerase chain reaction (PCR) followed by the detection through the low ionic-strength single strand conformational polymorphism (LIS-SSCP) electrophoretic technique. There were no significant differences in the allelic and genotypic frequencies for alleles 2, 3, and 4 in relation to the Mitsuda test among patients and household contacts, nor between those with MB and PB forms. However, individuals with a negative lepromin response associated with genotypes 22 and 23 presented a 7- and 8-fold greater chance of developing leprosy, respectively. Therefore, the NRAMP1 gene promoter polymorphism exhibited an interaction with the lepromin response, suggesting that allele 2 of the NRAMP1 promoter is an independent genetic factor that predisposes cells to enable pathogen survival, probably due to its low efficiency in iron transport. However, establishment of the infection and disease development may be conditioned by other immunological and genetic factors.

RéSUMÉ

Des résultats controversés ont été obtenus lorsque la relation entre le gène NRAMP1 et la susceptibilité à Mycobacterium leprae ou bien la réaction de Mitsuda, qui représente une réponse immunitaire spécifique contre M. leprae, a été étudiée. Cette étude a évalué l’association et également l’interaction entre le polymorphisme (GT)_n de la région promotrice du gène NRAMP1 et la réaction immunitaire spécifique contre M. leprae, mesurée par le test intradermique de Mitsuda chez des patients lépreux et chez des personnes non-consanguines en contact de ces patients. Le but de cette étude était d’évaluer l’association de ce polymor-
Leprosy, caused by *Mycobacterium leprae*, is a chronic disease that afflicts over 620,000 new cases per year, and most of these patients are found in India and in Brazil (38). The expression of the disease results from the interaction between the bacillus and the immunological system in such a way that most people infected develop an effective immune response against the bacillus without the presence of the disease, while others exhibit a spectrum of clinical manifestations intimately related to the immunological patterns of the host response to the pathogen (11, 12).

At one end of the clinical spectrum is tuberculoid leprosy (TT), in which bacterial growth is limited by a vigorous cellular immune response with a predominance of the CD4+ cells and the Th1-type cytokines (IL-2 and INF-γ) in the lesions of the skin. At the other end of the clinical spectrum is lepromatous leprosy (LL), in which bacterial growth is limited by an impaired cellular immune response with a predominance of the CD8+ cells and the Th2-type cytokines (IL-4 and IL-10) in the lesions of the skin.

The expression of the disease results from the interaction between the bacillus and the immunological system in such a way that most people infected develop an effective immune response against the bacillus without the presence of the disease, while others exhibit a spectrum of clinical manifestations intimately related to the immunological patterns of the host response to the pathogen (11, 12).

RESUMEN

Cuando se ha tratado de asociar el gene de susceptibilidad a *Mycobacterium leprae* NRAMP1, con la reacción de Mitsuda, se han encontrado resultados controversiales. La reacción de Mitsuda representa una respuesta inmune específica hacia *M. leprae*. En este estudio se evaluó tal asociación y la interacción del polimorfismo *(GT)*n en la región promotora del gene NRAMP1 con el resultado de la reacción de Mitsuda en pacientes con lepra y en contactos domésticos no consanguíneos. El objetivo del estudio fue evaluar la asociación de este polimorfismo génico con la resistencia o susceptibilidad a la enfermedad, y/o con la forma clínica de la misma en una población de una área endémica de Brasil atendida por el Centro Estatal de Referencia en Dermatología Sanitaria y Leprosa de la Universidad Federal de Uberlandia, MG. Los pacientes con lepra (90), diagnosticados de acuerdo a los criterios de Ridley y Jopling, se agruparon como multibacilares (MB) y paucibacilares (PB). El grupo control consistió de 61 contactos domésticos no consanguíneos. Los genotipos del promotor de NRAMP1 se obtuvieron por la reacción en cadena de la polimerasa (PCR) y por la técnica electroforética LIS-SSCP (low ionic-strength single strand conformational polymorphism).

No se observaron diferencias significativas en las frecuencias alélicas y genotípicas para los alelos 2, 3, y 4, en relación a la prueba de Mitsuda entre los pacientes y contactos domésticos, ni entre los pacientes con las formas MB y PB. Los individuos con respuesta negativa a la lepromina asociados a los genotipos 22 y 23 presentaron, respectivamente, 7- y 8-veces más probabilidad de desarrollar la enfermedad. Por lo tanto, observamos que el polimorfismo en el gene promotor de NRAMP1 presentó una interacción con la respuesta a la lepromina, sugiriendo que el alelo 2 del promotor de NRAMP1 es un factor genético independiente que predispone, en las células, la supervivencia del patógeno, probablemente debido a su baja eficiencia en el transporte de hierro; sin embargo, el establecimiento del bacilo y el desarrollo de la enfermedad pueden ser condicionadas por otros factores inmunológicos y genéticos.
opposite end is lepromatous leprosy (LL), in which the cytokine pattern found in the lesions is of the Th2-type (IL-4, IL-5 and IL-10), and CD8+ cells occur predominantly, along with a strong but inefficient humoral response (19, 40).

The Mitsuda test, which consists of the intradermal injection of a suspension of heat-killed \textit{M. leprae}, has been used as a measure of the cell-mediated immune response to the bacillus. The injection of the bacillus produces a positive local intradermal reaction in patients with tuberculoid leprosy, while lepromatous patients do not develop an intradermal reaction in response to the bacillus. From a clinical standpoint, the test is an important indicator of cellular immunity to \textit{M. leprae}. In patients with leprosy it is considered to be of good prognostic value for resistance when positive, and for susceptibility when negative, and in normal individuals a positive test is associated with a smaller risk of developing the disease (15). Although individuals with a negative Mitsuda response present a higher risk of developing lepromatous leprosy, some may never develop the disease, demonstrating that the relationship between Mitsuda reactivity and resistance is not fully established (15).

The origin of the inefficient immune response in individuals with multibacillary leprosy is uncertain, but the high rate of correspondence of leprosy type between identical twins favors the hypothesis of an association with genetic factors (16). The polymorphisms of the major histocompatibility complex (MHC) (20) and other genes not related to the MHC, such as the gene of the macrophage protein associated with natural resistance (NRAMP1, now known as \textit{SLC11A1}) (9, 21), vitamin D receptor gene (VDR) (31), TAP1 and TAP2 genes (25), have been associated with susceptibility to leprosy. Other loci, such as the tumoral necrosis factor (TNF) gene, linked to the HLA region, have also been reported to be able to determine the subtypes of leprosy (32, 34).

The NRAMP1 gene is composed of 15 exons and is located on human chromosome region 2q35, and covers at least 16 kb of DNA. It encodes a protein of 550 amino acids, the human Nramp1 integral membrane protein, that is found exclusively in the lysosomal compartment of monocytes and macrophages (6).

Over 10 polymorphic sites have been described in the NRAMP1 gene. In the promoter region of this gene, whose polymorphisms are functional since they affect the expression of the Nramp1 protein, 4 alleles were identified (1, 2, 3, and 4) (5). Alleles 2 and 4 are described as poor promoters of NRAMP1 and consequently, could offer protection against auto-immune diseases but would increase the susceptibility to infections by intracellular parasites. Allele 3 could yield larger expression of the Nramp1 protein, favoring protection against intracellular parasites, but it could also be involved in auto-immune syndromes (6).

Various studies have been carried out in an attempt to establish a connection between the polymorphisms of the NRAMP1 gene and susceptibility/resistance to auto-immune diseases (4, 7, 29, 36) and to diseases caused by parasites, such as \textit{Salmonella typhimurium}, \textit{Leishmania donovani}, and \textit{Mycobacterium bovis} (37).

One of the strategies to identify genomic regions associated with disease-causing genes is through genetic linkage studies based on a statistical estimate of whether two loci are likely to lie near each other, called the LOD score (decimal logarithm of an odds ratio: the odds that two loci are linked with recombination fraction divided by the odds that the two loci are unlinked, recombination fraction 0.5) (10, 22). In the traditional LOD scores, the boundary value of 3 it has been used to indicate linkage, and –2 for exclusion. Another strategy is the use of odds ratio (OR) and its confidence interval (CI) to indicate a chance for an event occurrence based on the variation of two categorical variables, with values of 0 and 1 (negative and positive results). The odds ratio can be interpreted as a measure of the magnitude of association between two raters. Calculating the standard error of log (OR), one can easily test the significance of log (OR) and/or construct confidence intervals (2).

Using LOD scores, haplotypes of the NRAMP-1 gene promoter region were associated with susceptibility to tuberculosis (6). Linkage studies between resistance to leprosy and polymorphisms of the NRAMP1 gene have not found any association of gene...
haplotypes with susceptibility to the disease in the French Polynesia (30). However, a co-segregation pattern was observed between the two proximal markers to the NRAMP1 gene, a microsatellite and a single nucleotide polymorphism (SNP), and susceptibility to leprosy (1). On the other hand, a linkage between the NRAMP1 gene and a positive reaction to the Mitsuda test was demonstrated in pairs of consanguineous individuals, regardless the presence or absence of leprosy (3). Another similar study has found no association between this gene and the Mitsuda test (16).

Consequently, the results obtained in the latest studies proved to be insufficient for an implication of the NRAMP1 gene with the susceptibility and/or resistance to leprosy, making it important to conduct further studies that may confirm the influence of this gene. This study evaluated the association as well as the interaction of the polymorphism \((GT)\_n\) in the promoter region of the NRAMP1 gene with a specific immune response to the \(M.\) leprae measured by the intradermal Mitsuda test, in leprosy patients and in non-consanguineous household contacts, aiming to evaluate the association of this gene with resistance or susceptibility to leprosy, and/or with clinical form of leprosy.

**SUBJECTS AND METHODS**

**Subjects.** Leprosy patients (90) and their household contacts (61) from the State Reference Center of Sanitary Dermatology and Leprosy of the Federal University of Uberlândia, (UFU), Minas Gerais (MG), Brazil, were invited to take part in this study, under the approval of the UFU Research Ethical Committee. Leprosy patients were diagnosed and classified according to the criteria of Ridley and Jopling (28), considering: clinical examination, histopathology of skin lesions, Mitsuda test, and the bacilloscopical index (BI) (27). Patients with undetermined types of leprosy were not considered.

For genetic analysis, we have used an adaptation of the WHO classification of patients (39), without considering the number of lesions, but taking into account the lesions characteristics and the bacilloscopic index. Therefore, patients were grouped into paucibacillary forms (45 patients), which consisted of tuberculoid (TT) and borderline-tuberculoid (BT) patients with negative bacilloscopy, and into multibacillary forms (45 patients), with mid-borderline (BB), borderline-lepromatous (BL) and lepromatous (LL) patients, who had a positive bacilloscopy.

The group considered as control was ensured by careful dermatological examination, and was composed of non-consanguineous household contacts who had similar exposure to \(M.\) leprae, but without symptoms or signs of the disease.

**Mitsuda test.** The Mitsuda antigen (suspension containing \(6.0 \times 10^7\) bacilli/ml, heat-killed), supplied by the Instituto Lauro de Souza Lima (ILSL, Bauru—SP), was injected intradermally (0.1 ml) at the upper third of the anterior face of the right forearm. The readings were performed by an experienced leprosy specialist 28 days after inoculation. The results were measured in millimeters and grouped for quantitative and qualitative analysis (15). However, for a genetic analysis using categorical data, all
patients and contacts were classified into two classes, according to their response pattern: “negative” for readings <7 mm, which consisted of negative and weak positive (+) reactions; and “positive” for readings ≥7 mm, which consisted of positive (++) and strong positive (+++) reactions or with the presence of fluctuant swelling and/or ulcerations.

Genotyping for the polymorphism on the NRAMP1 promoter gene. The DNA was extracted using the phenol:chloroform method described previously (33), with some modifications, and its quality was analyzed in agarose gels stained with ethidium bromide.

The amplifications through the PCR used the direct primer, 5′-CTCGCATAGGC-CAACGA and reverse 5′-TTCTGTGC-CTCCCAAGTTAGC (31). The conditions for PCR were 35 cycles at 95°C for 40 sec, 58°C for 40 sec and 72°C for 50 sec, preceded by initial denaturation of 95°C for 5 min and final extension at 72°C for 10 min. The reactions occurred were performed in a volume of 25 µl containing from 50 to 200 ng of genomic DNA, 1.5 mM of MgCl2, 200 µM of dNTPs, 7 pmol of each primer and 1.5 U of Taq DNA polymerase (Invitrogen, Carlsbas-CA).

The alleles 2, 3, and 4, containing 200, 198, and 188 base-pairs, respectively, were visualized in PAGE LIS-SSCP gel, 14% acrylamide:bis (49:1), at room temperature, with 10 V/cm for 24 hrs, followed by silver nitrate staining (Fig. 1). All polymorphic bands, representing all three alleles, were excised from the gel and cloned (TOPO-TA Cloning kit, Invitrogen, Carlsbas-CA) for confirmation of allelic sizes through dideoxy cycle sequencing. Allele 4 was also confirmed by the molecular weight previously described (13). The LIS-SSCP strategy allowed a fast and reliable characterization of the (GC)n promoter variation of the NRAMP1 gene, without using radioactivity and/or restriction endonucleases.

Statistical analysis. The statistical analyses were developed in SAS version 6.11 (1993) and Prophet version 5.0 (1996) programs. Comparisons of allelic frequencies of the NRAMP1 promoter polymorphisms among groups were evaluated by the t test. The Hardy-Weinberg Equilibrium for the NRAMP1 promoter polymorphisms in the population was tested by the χ² test. Regression analysis was performed to determine the association among all possible alleles and the Mitsuda test. Results of Mitsuda test means among groups were evaluated across NRAMP1 genotypes through analysis of variance and t tests. The association of the NRAMP1 genotypes and the Mitsuda test was verified by Pearson’s correlation and the χ² test. Odds ratios were determined for each genotype, Mitsuda tests (negative versus positive) and their interaction, comparing controls versus leprosy patients, to calculate the chance of developing leprosy.

RESULTS

The allelic frequencies of the polymorphisms for the NRAMP1 gene promoter analyzed by the chi-square test were not significantly different among groups (household contacts, paucibacillary and multibacillary patients) (Table 1). Frequencies of alleles 2 and 3 have been described elsewhere (6) to be 0.25 and 0.75, respectively. However, in this study, allele 2 presented higher frequencies, which varied from 0.32 to 0.36.

### Table 1. Allelic frequencies of the NRAMP1 gene promoter polymorphisms in leprosy patients, classified in paucibacillary and multibacillary forms, and household contacts of the State Reference Center of Sanitary Dermatology and Leprosy, UFU/SUS, Uberlândia, Minas Gerais, Brazil.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Allelic frequency</th>
<th>Allelic frequency</th>
<th>Allelic frequency</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household contacts</td>
<td>0.369</td>
<td>0.574</td>
<td>0.057</td>
<td>61</td>
</tr>
<tr>
<td>Paucibacillary patients</td>
<td>0.333</td>
<td>0.600</td>
<td>0.067</td>
<td>45</td>
</tr>
<tr>
<td>Multibacillary patients</td>
<td>0.322</td>
<td>0.656</td>
<td>0.022</td>
<td>45</td>
</tr>
</tbody>
</table>

N = number of subjects.
TABLE 2. Mean and standard deviation of the Mitsuda test result for leprosy patients and household contacts, according to the NRAMP1 promoter genotype, State Reference Center of Sanitary Dermatology and Leprosy, UFU/SUS, Uberlândia, Minas Gerais, Brazil.

<table>
<thead>
<tr>
<th>NRAMP1 genotype</th>
<th>Mean and standard deviation of the Mitsuda test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC PB patients MB patients Total</td>
</tr>
<tr>
<td>22</td>
<td>8 6.6 2.2 4 6.3 6.3 4 0.8 1.5 16 5.1 4.1</td>
</tr>
<tr>
<td>23</td>
<td>24 8.6 3.4 18 5.6 2.9 20 0.4 1.0 62 5.2 4.4</td>
</tr>
<tr>
<td>33</td>
<td>22 6.5 3.6 17 6.1 3.8 20 0.0 0.0 59 4.2 4.3</td>
</tr>
<tr>
<td>24/34/44</td>
<td>7 7.0 4.2 6 8.7 2.7 2 0.0 0.0 15 6.7 4.3</td>
</tr>
<tr>
<td>Total</td>
<td>61 7.4 3.5 45 6.3 3.7 46 0.2 0.8 152 4.9 4.3</td>
</tr>
</tbody>
</table>

N = number of patients or contacts; M = mean; S.D. = standard deviation in millimeters; HC = healthy contacts; PB = paucibacillary; MB = multibacillary. Differences in t tests were the following: HC vs. PB (t = 1.56; p = 0.06); HC vs. MB (t = 4.86; p <0.00001), and PB vs. MB (t = 10.8; p <0.00001).

Allelic and genotypic frequencies presented no statistical significance compared to those described in a previous study (37). However, significant differences were detected between alleles 2 and 3 within each group evaluated (p = 0.0017), with significantly lower frequencies of allele 2, 0.369, 0.333, and 0.322, for household contacts, PB and MB patients, respectively, in comparison to allele 3, which presented frequencies of 0.574, 0.60, and 0.656, respectively.

The mean value of Mitsuda test of household contacts (7.4) was greater than that observed in paucibacillary and multibacillary patients (6.3, and 0.2, respectively) supporting the association of disease resistance with the positive Mitsuda test. However, there were no differences among Mitsuda test means across NRAMP1 genotypes (Table 2). The Mitsuda test values were further transformed into qualitative results, classifying groups as negative and positive, as described. No association was found between NRAMP1 promoter genotypes or alleles and the Mitsuda test through regression analysis, using either quantitative or qualitative data. However, an interaction between them favoring leprosy occurrence was demonstrated through odds ratio analysis (Table 3).

Analysis of Mitsuda test results among household contacts (HC), PB and MB patient groups revealed significant differences (p <0.0001), except between contacts and PB, which revealed no differences (p = 0.06) (Table 2).

The bacterial index (BI) results for patients were compared among all NRAMP1 gene promoter alleles and no significant differences were detected for this variable among NRAMP1 promoter genotypes.

Odds ratios were calculated for each genotype in all combinations considering the leprosy patients and control groups, and very low values (under 1.0) were obtained, with non-significant statistical confidence intervals. The odds ratio for negative Mitsuda and disease status (leprosy occurrence) was 4.65 (χ² = 17.26; p <0.0001).

The lack of association between NRAMP1 genotypes and the Mitsuda test may lead us to consider them as two independent events. Therefore, the interaction of the NRAMP1 promoter genotypes and the Mitsuda response was further investigated, considering these as independent factors that could interact favoring disease establishment. The odds ratios were obtained for each genotype, comparing the Mitsuda response between leprosy patients and household contacts (Table 3). Results were highly significant for genotype 23 (OR = 8.09; CI 95%: 2.55 to 25.64) and for the combination of genotypes 22 and 23 (OR = 7.06; CI 95%: 2.57 to 19.39) when individuals presented a negative Mitsuda test. Genotype 22 presented a very high odds ratio (7.0) with a marginal confidence interval close to significance.

DISCUSSION

The present study has investigated the polymorphism in the promoter region of the NRAMP1 gene due to the strong evidence that such variation may be associated with the regulation of the gene, which is directly
associated with susceptibility to infectious disease (6). The results have demonstrated that the prevalence of alleles 2, 3, and 4 of the NRAMP1 gene promoter among groups (household contacts, paucibacillary and multibacillary leprosy patients) of this endemic area was not significantly different from other populations, although the frequency of the unfavorable allele 2 was 7% to 11% higher than that observed elsewhere (6). The higher prevalence of allele 2 in this population may be an indication that the local environment could be favoring or selecting for a higher frequency of this allele. However, it is believed that there may be a balanced selection between alleles 2 and 3 due to their probable association to infectious and autoimmune disease susceptibility, respectively (6).

This study focused on the polymorphism in the promoter region of the NRAMP1 gene in order to investigate the hypothesis that this polymorphism would be associated with the degree of the expression of the NRAMP1 gene, as proposed by previous studies (6, 8), and that the higher expression of the NRAMP1 gene would result in a more vigorous response to the Mitsuda antigen, with corresponding protection to the multibacillary forms of leprosy. However, no correlation was obtained between NRAMP1 genotypes and the Mitsuda response in this study, corroborating with results of other studies (16, 31). Similarly, association was observed with the different forms of leprosy, as demonstrated previously (27). On the other hand, the 4-bp polymorphism in the NRAMP 3′-untranslated region, detected in another study (18), was not associated to leprosy per se, but it was associated with one form of leprosy. Therefore, the contradictory results regarding this polymorphism were not able to confirm the possible influence of the NRAMP1 polymorphisms on the clinical presentation of leprosy.

The first evidence indicating an association of the NRAMP1 gene with leprosy was obtained in a segregation study of consanguineous pairs in 20 ethnic Chinese and Vietnamese family groups, where a significant non-random segregation was found for the haplotypes of the NRAMP1 gene among the consanguineous pairs (1). Additionally, in the segregation analysis according to the LOD score, the NRAMP1 gene haplotypes were associated with susceptibility to tuberculosis (10) and with positive results of the Mitsuda test (3). The latter study (3), associated to a previous study of the same families (1), has demonstrated a linkage of the chromosome 2q35 locus with Mitsuda response, which has only identified a candidate region where a gene is located near or at the locus controlling the Mitsuda response. However, in that investigation (3), a possible confounding effect of disease status was shown on the linkage test, which is highly influenced by the Mitsuda response. It is also important to notice that the small data set of consanguineous sib-pairs may have suffered an im-

### Table 3.

<table>
<thead>
<tr>
<th>Genotype(s)</th>
<th>Groups</th>
<th>Mitsuda test (number of individuals)†</th>
<th>Odds Ratios (CI = 95%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>22</td>
<td>Control</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Leprosy</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>23</td>
<td>Control</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Leprosy</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>33</td>
<td>Control</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Leprosy</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>22 + 23</td>
<td>Control</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Leprosy</td>
<td>10</td>
<td>37</td>
</tr>
</tbody>
</table>

† Mitsuda test results: positive (37 mm) and negative (<7 mm).
‡ Odds ratio followed by * indicates statistical significance. CI = confidence interval.
important genetic bias due to the allele fixation in a very small number of families, which favors allele coincidence, where sibs have a 75% probability of sharing at least one allele. This is supported by the ethnic background: significant differences were observed only for the Vietnamese population (3), but not for Chinese (3), Brazilian (16), and Indian (31) populations. Since the power of LOD tests for establishing linkage or exclusion is decreased with small data sets, as demonstrated elsewhere (24), it is possible that the linkage shown with the quantitative Mitsuda trait (3) may be only a trend which indicates a possible interaction with other candidate immunological and genetic factors. Another important confounding effect is the period and frequency of contact with infected patients and the time required for disease development, which can not be recorded or accurately estimated, producing a false positive linkage due to genotypic disequilibrium in small populations. Contradictory Mitsuda test results may further complicate this analysis due to the presence of positive Mitsuda individuals that develop disease, as well as negative Mitsuda individuals that will never develop leprosy, suggesting that other factors may be contributing to the disease development. In fact, there are very few linkage studies firmly established for quantitative traits in humans, especially because statistical methods for exclusion are still underdeveloped (24).

In our study, we have genotyped 90 patients and 61 non-consanguinous household contacts. This population sample is a true representation of allelic and genotypic frequencies of the Brazilian population that is in Hardy-Weinberg equilibrium. Differences between our study and results obtained elsewhere (3) are related to the consanguinity status and the number of patients representing families. Our study represents 90 different families, while the earlier studies represent 20 nuclear families. However, the allelic and genotypic frequencies distribution in these two populations could not be compared since no genotypic information was provided on the study with 4 Chinese and 16 Vietnamese families (3), nor was possible to verify the frequency of the other microsatellite loci and their linkage with the Mitsuda tests.

An earlier study of 30 individuals (22 healthy and 8 leprosy patients) did not detect any association of the loci 274C/T, D543N and 1729 of the NRAMP1 gene with the positive reaction to the Mitsuda test (16). Additionally, the LOD score tested in 7 family groups in French Polynesia, including 39 leprosy patients and 45 healthy individuals, was not significant for NRAMP1 haplotypes and leprosy association (20). Another investigation also failed to detect an association of the promoter \((GT)^n\) region, the 274C/T and the TGTG deletion with the lepromatous and tuberculoid forms of leprosy (31). All these observations are in agreement with this investigation, which has not found any association between NRAMP1 promoter polymorphisms with leprosy \textit{per se}, nor with Mitsuda tests or with leprosy types. However, the NRAMP1 gene may have influence over the pathogenicity of leprosy, since there is increasing evidence favoring the linkage of the locus NRAMP1 with tuberculosis, which is also a disease in which macrophage infection is important (14).

Although there is a high association of the positive Mitsuda test with leprosy resistance, the high variability of these tests in household contacts and PB patient’s groups observed in this study supports the hypothesis that resistance to leprosy is conditioned by multiple genes (23, 26), and these genes may be masking the true effect of the NRAMP1 gene in the disease outcome.

No interaction between Mitsuda tests and NRAMP genotypes has previously been investigated relating the two variables to leprosy. This is the first study that uses combined data of NRAMP1 genotypes and Mitsuda tests classes to estimate the chance of developing leprosy. The odds ratio for negative Mitsuda and leprosy occurrence was highly significant (OR = 4.65) as shown elsewhere (15) corroborating the close association of negative Mitsuda results and leprosy susceptibility. On the other hand, the odds ratio for allele 2 and leprosy occurrence was non-significant (OR = 0.88; \(p > 0.05\)).

However, in this study we have also shown a very important interaction between NRAMP1 gene and Mitsuda tests. The lack of association between the two variables may lead us to consider them as two independent events that may interact with each
other. The NRAMP1 promoter genotypes 22 and 23 were found to be unfavorable genotypes when present in combination with a negative Mitsuda response, showing an approximately 7-fold greater chance of developing leprosy disease. The high odds ratio for genotype 22 (7.0) with a confidence interval not quite reaching significance (probably due to the low number of individuals evaluated in this class, provides an indication that this genotype may also present the same significant tendency of genotype 23) suggests that a dominance of allele 2 favors pathogen survival. Consequently, the susceptibility phenotype could be determined by the association between autologous factors such as low-activity of the NRAMP1 gene promoter alleles, point mutations and/or deletions with influence upon the transporting function of iron ions of the NRAMP1 protein, and several exogenous factors. These results are supported by other studies (20, 21) which suggest that susceptibility to leprosy is multigenic, with a high heterogeneity among different populations studied. In this sense, a complex genetic model of susceptibility to leprosy could make detection of linkage difficult (1).

Recently, a complex segregation analysis and a genome-wide scan have demonstrated that the susceptibility to the disease itself or its progression may be related to different genetic factors. Siddiqui, et al. (35) identified a major susceptibility locus to leprosy (10p13) regardless of the polygenic nature of the disease. On the other hand, it was demonstrated that the segregation of the HLA/TNF region, locus 6p21, has a strong link to the development of the clinical forms of leprosy (21).

Mira, et al. (20) proposed a genetic model for leprosy susceptibility in two phases: susceptibility of the disease itself would be linked with non HLA genes such as the NRAMP1, in other words, genes that would control the progress of the infection until the development of the clinical symptoms of the disease, while the loci linked to the HLA would determine the subtypes of leprosy.

The hypothesis that the differential NRAMP1 gene expression is related to M. leprae survival (6) instead of microbial proliferation (17) is supported by this study that demonstrated that there was no association of the NRAMP1 promoter genotypes and the bacilloscopic index. Hence, the low expression of the iron transport protein (NRAMP1), conditioned by the allele 2, may function in microbial persistence, especially in Mitsuda-negative individuals.

We propose that the NRAMP1 gene favors microbial survival, probably by transporting the iron inefficiently, but it will only affect the development of clinical symptoms in association with other immunological and genetic factors, as demonstrated by the interaction with a negative Mitsuda test. Therefore, leprosy susceptibility cannot be conditioned by a unique gene; instead, it is a function of multifactorial host conditions that require at least two independent molecular events that interact to each other.

Acknowledgment. We thank Dr. Maria Ester Sales Nogueira to have kindly given the Mitsuda reagents and the staff of the State Reference Center of Sanitary Dermatology and Leprosy of the Federal University of Uberlândia (UFU) for fundamental support.

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A Mutation at Codon 516 in the \textit{rpoB} Gene of \textit{Mycobacterium leprae} Confers Resistance to Rifampin\textsuperscript{1}

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\textbf{ABSTRACT}

A missense mutation at codon 516 in the \textit{rpoB} gene of \textit{Mycobacterium leprae} conferring rifampin resistance was confirmed by the correlation between sequencing results and mouse footpad assay. The isolate was obtained from a relapsed lepromatous leprosy patient. This is the first report on the complete concordance between the mutation located at codon 516 in the \textit{rpoB} gene and the corresponding resistance to rifampin in leprosy. The novel profile of mutation in the \textit{rpoB} gene will contribute to the comprehensive understanding of rifampin resistant patterns and offer a useful tool for developing simple and rapid drug susceptibility testing approaches, which would promise more effective and successful control of leprosy.

\textbf{RÉSUMÉ}

Une mutation faux-sens localisée au codon 516 du gène \textit{rpoB} de \textit{Mycobacterium leprae} a permis l’expression d’une résistance à la rifampicine, qui a été confirmée par une corrélation entre les résultats du séquençage et le test d’inoculation à la patte de souris. L’isolat a été obtenu à partir d’un patient souffrant de lèpre lépromateuse et qui a rechuté. Ceci est le premier article rapportant une concordance complète entre la mutation localisée au codon 516 du gène \textit{rpoB} et une résistance à la rifampicine dans le contexte de la lèpre. Ce nouvel éventail de mutation du gène \textit{rpoB} va contribuer à une compréhension plus complète des alternatives de résistance à la rifampicine. Il devrait offrir un outil utile au développement d’approches pour le test simple et rapide de la résistance à la rifampicine, qui devrait résulter en un contrôle plus efficace et réussi de la lèpre.

\textbf{RESUMEN}

Una mutación sin sentido en el codón 516 del gene \textit{rpo B} de \textit{Mycobacterium leprae}, que le confiere resistencia a la rifampina, fue confirmada por correlación de los resultados de la secuenciación y del ensayo en la almohadilla plantar del ratón. La cepa fue obtenida de un caso de recaída de lepra lepromatosa. Este es el primer reporte sobre la concordancia perfecta entre la mutación localizada en el codón 516 del gene \textit{rpo B} y la resistencia a la rifampina en la lepra. El nuevo perfil de mutación en el gene \textit{rpo B}, aparte de que ayudará a entender los patrones de resistencia a la rifampina, constituye una nueva herramienta para el desarrollo de métodos simples y rápidos para probar la susceptibilidad de la bacteria a la droga, lo cual seguramente contribuirá al control exitoso y efectivo de la lepra.

\textbf{RESUMEN}

Rifampin is a key component of multi-drug therapy (MDT) suggested by the World Health Organization (WHO) in the treatment of leprosy (\textsuperscript{19}). Resistance to rifampin often gives rise to treatment failure, which subsequently threatens the effective control of leprosy (\textsuperscript{1,8}). However, resistance to rifampin has been constantly documented since 1976, by using standard mouse footpad assay (\textsuperscript{2,4,12,13,15,17}). For this reason, it is suggested that clinically suspected relapsed leprosy cases and those who exhibit an unsatisfactory response to antileprosy therapy should be subjected to drug susceptibility testing. Understanding of drug resistance is essential for the effec-
tive treatment and control of leprosy. Therefore, simple and rapid methods for drug resistance testing are necessary. To establish such methods, solid basic data for the correlations between mutation and phenotype of drug resistance are required. In the present study, an isolate obtained from a relapsed Japanese leprosy patient was investigated for drug susceptibility testing by both genetic analysis and the standard mouse footpad method. It has been assumed that mutations which cause rifampin resistance in *Mycobacterium tuberculosis* are almost the same as in *M. leprae*. Although it has been confirmed that mutations at codon 513, 526, 531, and 533 in the *rpoB* gene of *Mycobacterium leprae* confer rifampin resistance, no substantial evidence shows whether a mutation at codon 516 relates to rifampin resistance or not. The goal of this report was to confirm the missense mutation at codon 516 in the *rpoB* gene conferring rifampin resistance.

**MATERIALS AND METHODS**

*M. leprae* isolate. The isolate, named as Kusatsu-6, was detected in a skin biopsy sample obtained from a 75-yr-old Japanese lepromatous leprosy male patient. The patient had been treated with dapsone monotherapy for 18 yrs, and then with rifampin alone for 10 yrs before another 14 yrs monotherapy of dapsone. The patient relapsed and, because he was considered likely to have taken his medicine irregularly and had had long-term monotherapy, he was suspected of harboring drug-resistant *M. leprae*.

Drug susceptibility testing in the mouse footpad. The biopsy specimen was processed to recover *M. leprae* in the same manner as previously described (12). The initial bacillary suspension containing 1.0 x 10^6 in 0.05 ml of Hank’s balanced salt solution (HBSS) was inoculated into the hind footpads of BALB/c-*nu/nu* mice since the viability of the bacilli in the material treated with antileprosy drugs was unknown. Approximately 12 months after inoculation, bacterial suspension for drug susceptibility testing was prepared from the nude mice footpads, which had shown bacillary multiplication. Drug susceptibility testing, for dapsone, rifampin, ofloxacin, sparfloxacin, clofazimine, and clarithromycin was performed in the same manner as previously presented (11, 12, 13). Additionally, 0.08% minocycline (1) was added to the drug group for susceptibility testing in the present study. Bacillary growth in the mice footpads was examined after treatment with each drug for 25 weeks (17).

Genetic analysis for mutation. Sequencing was conducted as previously reported (11). Briefly, the initial biopsy suspension of Kusatsu-6 was partially purified by differential centrifugation and the pellet was resuspended in 50 µl of lysis buffer consisting of Proteinase K and Tween 20, then incubated at 60°C for 18 hr, followed by snap freeze-heating (~84°C for 30 min and then 98°C for 10 min) to extract the genomic DNA and inactivate proteinase K.

Primers with the following sequences were used: folP F 5’GCT TCT CGT GCC GAA GCG CTC3′ and folP R 5’GCC ATC GCG GGA TCT GCT CGC CCA3′; rpoB F0 5’CAG GAC GTC GAG GCG ATC AC3′ and rpoB R0 5’CAG GAC GTC GAG GAC TCG ATC AC3′ and gyrA FN 5’CAG GAC GTC GAG GCG ATC AC3′ and gyrA RN 5’TAC CCG GCC AAC CGA AAT TG3′. These amplimers target a 388-bp fragment of the folP gene, a 382-bp fragment of the rpoB gene and a 342-bp of the gyrA gene in *M. leprae*, which contain mutations corresponding to dapsone-, rifampin-, and quinolone-resistance, respectively. DNA was amplified by G mixture of FailSafe PCR System (EPICENTRE, Madison, WI. U.S.A.), the amplified product was verified by electrophoresis and recovered by using MinElute Gel Extraction Kit (QIAGEN, GmbH, Germany). The sequencing reaction was performed by the BigDye Terminator Cycle Sequencing FS Ready Reaction kit (Perkin-Elmer Applied Biosystems, Norwalk, CT, U.S.A.). The sequencing reaction was performed by the BigDye Terminator Cycle Sequencing FS Ready Reaction kit (Perkin-Elmer Applied Biosystems, Norwalk, CT, U.S.A.). Direct sequencing of the PCR products was performed with the ABI Prism 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Norwalk). Sequencing data was analyzed by the DNASIS program (Hitachi Software Engineering, Yokohama, Japan), as presented elsewhere (9, 11, 14). The DNA sequence was compared with that in the GenBank database.

**RESULTS**

Drug susceptibility in the mouse. Bacillary growth in mouse footpads administered
0.01% rifampin, 0.01%, 0.001% and 0.0001% dapsone showed almost the same level of growth as observed in the control mice. No bacillary growth was noticed in footpads of mice treated with ofloxacin, sparfloxacin, clofazimine, clarithromycin, and minocycline (Fig. 1). According to the results of this mouse footpad assay, Kusastu-6 was concluded to be resistant to rifampin and dapsone at high concentration, but susceptible to the other drugs mentioned above.

**Genetic analysis.** The expected PCR products of the *folP*, *rpoB* and *gyrA* gene was successfully obtained from Kusatsu-6. The sequencing results displayed a missense mutation in the *rpoB* gene, affecting the codon at position 516 (numbering system applied for *E.coli*), GAT → TAT, leading to an amino acid substitution, Asp → Tyr, simplified as Asp-516-Tyr (Fig. 2). Similarly, a missense mutation at codon 55 (CCC → CTC, Pro-55-Leu) in the *folP* gene was revealed. No mutation was found at codon 89 or 91 in the *gyrA* gene.

**DISCUSSION**

Single point mutations within an 81-bp region in the *rpoB* gene involving 5 codons, Gly-513, Asp-516, His-526, Ser-531 and Leu-533 have been proved to lead to rifampin resistance in *Mycobacterium tuberculosis* (16, 20). The deduced amino acid sequence of this region presented 100% identity to that in *M. leprae* (22). According to the highly conserved nature of this region in the *rpoB* gene, six distinct mutations affecting 4 codons (Gly-513, His-526, Ser-531 and Leu-533) within this region carrying resistance to rifampin have been already clarified in *M. leprae* (1, 5, 11, 13, 22). Nevertheless, until now there has been direct evidence to explain whether the mutation at codon Asp-516 in the *rpoB* in *M. leprae* is linked to rifampin resistance, even if in *Mycobacterium tuberculosis* mutation at this codon Asp-516 confers rifampin resistance. To our knowledge, the present study is the first report to elucidate that this mutation at codon 516 is responsible for rifampin resistance in *M. leprae*.

As we know, the standard mouse footpad assay, which has been employed for drug resistance testing in leprosy for 40 yrs, requires not only long periods of at least 12 months to get results, but also requires considerable facilities, expertise and rigorous restrictions on the conditions of the *M. leprae* examined (7, 8). Therefore, it is necessary and urgent to establish rapid and routinely applicable approaches for the detection of drug resistance in leprosy. Recently, the characterization of the mutations at codons Gly-513, His-526, Ser-531 and Leu-533...
have been used to set up simple and feasible alternative protocols. PCR-single strand conformation polymorphism (PCR-SSCP) (6), PCR-heteroduplex formation assay (PCR-HDF) (22), solid-phase hybridization to oligonucleotide capture probes (7) and Touch-Down PCR (10) have yielded satisfactory preliminary evaluations in leprosy. On the other hand, a comprehensive understanding of mutations in the rpoB gene correlated with rifampin resistance enhances the reliability and integrity of promising methods for drug susceptibility testing. This novel profile of the mutation at codon Asp-516 contributes to data on mutation patterns of rifampin resistant M. leprae, and is certainly worthwhile in the determination of drug susceptibility testing in leprosy. The multiplication of Kusatsu-6 in mouse footpads treated by rifampin indicated the full concordance with the missense mutation of Asp-516-Tyr in the rpoB gene.

In addition, the molecular mechanisms of dapsone- and quinolone-resistant M. leprae have been adequately described so far. Dapsone-resistant relevant mutations are limited at codons 53 and 55 in the folP gene (9,21) whereas mutations reflecting resistance to quinolone affect codon 89 and 91 in the gyrA gene (11). Our results clearly demonstrated that the dapsone-resistant M. leprae harbored a missense mutation at codon 55 (CCC→CTC, Pro-55-Leu) in the folP gene. Mutations in the folP gene are commonly detected among Japanese relapsed leprosy cases because MDT was not applied to them until 2000, as we discussed previously (13). In spite of this, the lack of bacillary growth in the mice footpads administered ofloxacin and sparfoxicin was identical to the result of the gyrA sequencing. All the results presented complete agreement between in vivo susceptibility and genetic tests.

In conclusion, the verification of the mutation at codon Asp-516 in the rpoB gene is involved in rifampin resistance in M. leprae. This finding offers valuable information for molecular drug susceptibility testing in leprosy and hopefully will help to provide a useful tool for the further successful control of leprosy all over the world.

Acknowledgment. This study was supported by the grant of the Postdoctoral Fellowship Program, Japan Society for the Promotion of Science, and the grant of a Health Research Grant of Emerging and Re-emerging Infectious Diseases, Ministry of Health, Labor and Welfare, Government of Japan.

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![Fig. 2. rpoB sequencing result. (A) codon 516 GAT for amino acid Asp in wild type M. leprae. (B) codon 516 TAT for the substituted amino acid Tyr in Kusatsu-6.](image-url)
“Were-Wolf” Cutaneous Tuberculosis

Vikram K. Mahajan, Nand Lal Sharma, and Ramesh Chander Sharma

ABSTRACT

Lupus vulgaris is a variant of cutaneous tuberculosis. Its more destructive and mutilating clinical forms have become rarer in consonance of a general decline of cutaneous tuberculosis. It is rarely seen now in developed countries due to stringent control measures, improved quality of living and effective therapeutic regimens. Misdiagnosis, neglect, or late diagnosis may result in severe, ulcerative and mutilating “wolf eaten” skin lesions.

This paper describes four such cases of “were-wolf” cutaneous tuberculosis. Early diagnosis and treatment is important to prevent much of the disfigurement.

RÉSUMÉ

Le lupus vulgaire est la variante la plus commune de la tuberculose cutanée. Ses formes cliniques plus destructives et plus mutilantes sont devenues rares, en relation avec un déclin général de la tuberculose cutanée. Il est rarement observé maintenant dans les pays développés où il existe des mesures rigoureuses de contrôle, une qualité de vie améliorée et des traitements efficaces. Des erreurs de diagnostic, des négligences ou encore un diagnostic tardif peuvent résulter en des lésions cutanées ulcéreuses et mutilantes dites ‘en morsure de loup.’

Cet article décrit quatre de ces cas de tuberculose cutanée dite ‘de loup.’ Un diagnostic précoce et un traitement sont importants afin d’éviter que le patient ne soit défiguré.

RESUMEN

Lupus vulgaris es una variante común de la tuberculosis cutánea. Sus formas más destructivas y mutilantes han llegado a ser muy raras, en consonancia con la disminución general de la incidencia de la tuberculosis cutánea. Actualmente se observa raramente en los países desarrollados debido a las estrictas medidas de control, al mejoramiento en la calidad de vida y a los regímenes terapéuticos efectivos. El diagnóstico equivocado, el descuido, o el diagnóstico tardío, favorecen el desarrollo de lesiones dérmicas severas, ulcerativas y mutilantes, conocidas como “mordidas de lobo.”

Este artículo describe 4 casos de tuberculosis cutánea tipo “mordida de lobo.” El diagnóstico y tratamientos tempranos son importantes en la prevención de esta condición clínica desfigurante.

Lupus vulgaris (LV) is a chronic, progressive form of cutaneous tuberculosis occurring in individuals with a moderate to high degree of immunity. The characteristic lesion is a plaque composed of reddish brown nodules, which on diascopy reveals an “apple jelly” color. The disease process is usually associated with scarring and atrophy causing considerable tissue destruction over many years. The common clinical forms include papular, nodular, plaques, ulcerative and timid lesions but atypical morphology is becoming more common. Uncommon forms, such as frambosiform, gangrenous or ulcero-vegetating type of lesions too have been documented (3). The term “lupus” means wolf; perhaps the name alludes to the appearance of a face that has been chewed by a wolf (1). Such “wolf eaten” appearance is uncommon and seen only in the severe, ulcerative, mutilating type of lupus vulgaris of the face.

The more destructive and mutilating forms have become rarer, at least in immunocompetent patients, due to better awareness, effective treatment regimens
and better access to health care facilities. However, the diagnosis of LV may often be delayed when the index of suspicion is low, especially in the developed countries, where the disease has become very rare (15). As the disease has potential to mutilate when left untreated, leaving deforming scars and disfigurement, an early diagnosis is of paramount importance.

We present here four cases of this disease that is not so rare in developing countries where the importance of high clinical suspicion, early diagnosis and treatment can not be over emphasized.

**Case 1.** This 24-yr-old male patient presented with multiple, asymptomatic, and slowly progressive facial lesions and deformed nose. History revealed that about 10 yrs earlier he had developed a nodulo-plaque lesion over right submandibular area. Similar new lesions continued to appear all over the face in the next 4 to 5 yrs. He had also developed deformity of the tip of the nose and right eye during this period. There was no history of any major systemic illness any time during the course of his disease. For his cutaneous lesions, various medical practitioners had treated him at different times during his illness, but to no avail.

Cutaneous examination showed (Fig. 1) multiple, multicentric, papulo-nodulo-plaque lesions of variable size and shapes over both cheeks, the malar area of face, nose and upper lip. The lesions were discrete, but had coalesced at places to form bigger lesions. A few lesions also showed subtle, adherent scales. There was loss of both alae nasi (more loss on the left), contractures of the right upper eyelid with cicatricial ectropion, loss of eyelashes and partial destruction of lid margins. The eyelashes of the left eye were partially absent due to lesions over lid margins. Both nasolacrimal ducts were blocked resulting in epiphora. A few lesions showed healing with atrophic, paper-thin scars. Examination of the nasal cavity revealed partial destruction of the nasal septum and extension of facial lesions inside. There was no re-
Regional lymphadenopathy. Oral mucosa, hair, nails, and other systemic examination were normal.

Laboratory investigations, including complete blood counts, ESR, blood sugar, hepatorenal function tests, x-ray films for chest and paranasal sinuses, ultrasonography of abdomen, HIV serology, urinalysis, and sputum examination for acid-fast bacilli (AFB), did not show any abnormality. The Mantoux test was positive, measuring $15 \times 20$ mm. Histopathology from one of the lesions showed an unremarkable epidermis, a few epitheloid cell granulomas with Langhan’s giant cells in the dermis and at places small areas of necrosis. No acid-fast bacilli (AFB) could be demonstrated either in direct smears, histopathologic sections or from culture of biopsy specimen on Loewenstein-Jensen (LJ) medium.

Treatment with rifampicin 450 mg/day, isoniazid 300 mg/day, ethambutol 800 mg/day and pyrazinamide 1250 mg/day was effective within a month in checking the progress of the disease and further tissue destruction. This treatment was continued for one month more. During the continuation phase of the next four months the patient received only rifampicin and isoniazid. All the lesions healed completely but with disfiguring scarring.

**Case 2.** This 40-yr-old male patient was hospitalized with progressive loss of his nose tip associated with foul smelling discharge. He stated that a year earlier he had an erythematous, papulo-nodulo-plaque lesion over the tip of the nose associated with swelling of the nose and surrounding area. The lesion was progressive in size and had resulted in loss of tip of the nose during this period. He had no associated systemic symptoms at any time during the course of his disease. Treatment from local practitioners had not been helpful.

Cutaneous examination (Fig. 2) revealed destruction of the tip of the nose and nasal

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**FIG. 2.** Destruction of the tip of the nose and nasal septum.
septum, exposing a nasal cavity full of foul smelling exudate. There was erythema and edema of surrounding skin. An ill defined, erythematous, infiltrated, and locally crusted plaque involved the philtrum, nose, left cheek, and nasal bridge. A few small and isolated plaques were also present on the forehead and left infra-orbital area. There was no regional lymphadenopathy. Examination of oropharynx, hair, nails and other systems did not reveal any abnormality.

A high ESR (70 mm in 1st hr) and negative Mantoux test were the only abnormalities found in the various laboratory investigations carried out. Histopathology was consistent with lupus vulgaris. AFB could not be demonstrated in direct smears, histopathology or culture.

The patient was treated with rifampicin 450 mg/day, isoniazid 300 mg/day, ethambutol 800 mg/day, and pyrazinamide 1250 mg/day for the initial two months. Lesional erythema and induration subsided within the first month of treatment. The treatment with rifampicin 450 mg/day and isoniazid 300 mg/day was continued for next four months. All lesions healed with scarring and disfigurement at the end of treatment.

Case 3. This 16-yr-old girl was hospitalized with a large, ulcerated, crusted lesion involving the nostrils and lips, of four years duration. It had started as a small nodulo-plaque lesion over the upper lip that progressively enlarged to involve the nearby areas. She had no other associated systemic symptoms.

Cutaneous examination showed a large ulcerated lesion involving alae nasi, nostrils, and the entire upper and lower lips. It was covered with thick hemorrhagic crusts, with fissuring and a tendency to bleed. There was diffuse erythema, edema, and infiltration of surrounding skin extending irregularly beyond the central crusted lesion (Fig. 3). Examination of the oral cavity showed irregular, erythematous, granulating patches over the hard palate. The nasal cavity could not be examined due to exudates and bleeding from the lesions. The nasopharynx did not show any lesions.

Laboratory investigations did not reveal any abnormalities. The Mantoux test was positive, measuring 20 × 24 mm. Histopathology showed features of lupus vulgaris. AFB could not be demonstrated in any of the specimens.

Fig. 3. Infiltrated, fissured, crusted and hemorrhagic lesions of lupus vulgaris involving nostrils, lips and surrounding skin.
The lesions showed healing after four weeks of rifampicin 450 mg/day, isoniazid 300 mg/day, ethambutol 600 mg/day and pyrazinamide 1000 mg/day. After two months of intensive treatment with these drugs she showed further improvement. Treatment was continued for four months with rifampicin 450 mg/day and isoniazid 300 mg/day. Scarring and cicatricial microstomia remained afterwards.

Case 4. This 28-yr-old female patient presented to us with almost complete loss of right earlobe. It had started about 10 yrs prior, following a cosmetic ear piercing, which had developed into an asymptomatic, slowly progressing non-healing ulcerative lesion having intermittent purulent discharge. Slowly and steadily her earlobe was lost as the tissue eroded (Fig. 4).

Cutaneous examination revealed an absent right earlobe almost extending to the cartilage. The residual part had ragged edges, erythematous and granulomatous infiltration and crusting in places. There was no regional lymphadenopathy. Hair, nails, and mucous membranes were normal. There were no associated systemic symptoms. Systemic examination did not reveal any abnormality.

Laboratory investigations, carried out as in other cases, did not demonstrate any abnormality. Histopathology showed a normal epidermis, but the dermis had a few ill-formed epitheloid cell granulomas. The dense inflammatory lymphoplasmacytic infiltrate extended into and involved the subcutaneous tissue. Staining for AFB was positive. The Mantoux test could not be carried out due to non-availability of tuberculin at the time. Culture of the biopsy specimen on LJ medium did not show growth of mycobacteria.

She was lost to follow-up after initiation of antituberculosis treatment.

**DISCUSSION**

Cutaneous tuberculosis has declined after the introduction of highly effective antituberculosis treatment regimens. In an Indian study (11), cases of cutaneous tuberculosis comprised only 0.15% of all dermatology out-patients compared to previously re-
ported incidence of 0.25% and 0.59% \(^{(9,13)}\). Lupus vulgaris is the most common variant of cutaneous tuberculosis, accounting for nearly 59% of secondary skin tuberculosis cases in India with an average prevalence of 0.37% among general dermatology patients \(^{(7,12)}\). It has become so rare in USA that “Lupus,” unqualified, means lupus erythematosus and not lupus vulgaris \(^{(1)}\).

Lupus vulgaris is acquired exogenously by direct inoculation of the bacilli or endogenously via hematogenous or lymphatic spread from associated tuberculosis of other organs. Primary inoculation tuberculosis comprises 0.14% of all primary tuberculous lesions, i.e., tuberculous chancre and the primary tuberculous complex \(^{(14)}\). The latter is a cutaneous analog of as the Ghon complex primary pulmonary tuberculosis and evolves into inoculation lupus vulgaris. Although primary inoculation lupus vulgaris has been reported \(^{(8,10)}\) it is not very frequent. The disease shows a predilection for the face and almost 80% of the lesions are seen over the head and neck. On the face it tends to involve the nose, earlobes, upper-lip and frequently extends to the contiguous mucosal surfaces.

Nasal lesions start as nodules that bleed easily and then ulcerate, sometimes destroying the cartilage. Destruction of the whole nose may occur leaving only orifices and the posterior parts of septum and turbinates visible. The upper-lip, when involved, becomes swollen, thickened, and fissured having adherent crusts and a tendency to bleed. Granulating, vegetating, or ulcerative lesions of the buccal mucosa, palate, gingiva, or oropharynx may occur from direct extension or by lymphatic spread from nasal lesions.

Spontaneous healing may occur but not without scarring, atrophy, contractures, and tissue destruction. On the face, such mutilations and scarring leads to “parrot-beak” nose, ectropion and atrophied lips that may eventuate in a “were-wolf” appearance.

Sarcoidosis and rosacea may sometimes simulate early lupus vulgaris. Histopathology and tissue culture studies may also be required as clinical differentiation from several diseases is often difficult, including tertiary syphilis, chronic discoid lupus erythematosus, deep mycoses (sporotrichosis, blastomycosis, chromoblastomycosis), leishmaniasis, and—more commonly—leprosy. It is important that biopsies taken are deep enough to be representative.

Demonstration of a classical tubercular granuloma on histopathology is diagnostic but caseation necrosis is usually sparse or absent \(^{(1)}\). Demonstration of AFB in Ziehl-Neelson stained tissue smears, or their recovery in culture on LJ medium is disappointing in most instances. AFB were present in only 5% patients of lupus vulgaris in a recent study \(^{(6)}\). Scattered, non-caseating, compact epitheloid cell granulomas sparsely surrounded by lymphocytes are characteristic of sarcoidosis. Tuberculoid leprosy is differentiated from neural and perineural granulomatous inflammation. Tertiary syphilis shows more pronounced vascular changes and plasma cell infiltrates. Non-specific tuberculoid infiltrates without formation of typical tubercles is seen in rosacea. Demonstration of causative organisms in histologic sections or cultures will be diagnostic in leishmaniasis and deep mycoses.

The diagnostic value of a positive Mantoux test is ambiguous if the patient has had BCG vaccination or other mycobacterial exposure. A strongly positive test is significant but the sensitivity decreases with advancing age, early treatment, and conditions that reduce delayed hypersensitivity. Other than these factors and technical errors (e.g., subdermal injection of tuberculin), about 5% of patients do not react to ordinary intermediate strength of tuberculin used, for reasons unknown \(^{(4)}\).

Polymerase chain reaction (PCR) provides rapid, specific and sensitive testing for \(M. \) tuberculosis. In polymerase chain reactions (PCR), discrete fragments of DNA are specifically amplified from specimens in cutaneous tuberculosis. A specific \(M. \) tuberculosis genome fragment, mtp 40, has been identified that allows prompt differentiation from atypical mycobacteria \(^{(16)}\). The high cost and need of expertise, however, limit its routine use.

All of our patients exhibited classic features of lupus vulgaris. An early diagnosis and specific treatment would have prevented much of the mutilation. It is baffling that all these patients neglected themselves or could not be diagnosed for years especially in this era of diagnostic and therapeutic advancements. It seems that in the absence of symp-
Symptoms such disfigurement (even of the face) remains of little consequence in view of the high cost of treatment or specialized consultations. We feel that a high index of clinical suspicion is of foremost importance in the diagnosis of cutaneous tuberculosis during the early stage of the disease. This is particularly true for areas where the disease is seen rarely. Nevertheless tuberculosis remains a disease of great importance as it can be treated effectively.

In view of emerging drug resistant strains only multi-drug chemotherapy is now recommended for all forms of tuberculosis, and a strict compliance to the regimen is imperative. It needs no modification in dermatological practice. The standard regimen consists of rifampicin (10 mg/kg, up to 600 mg/day), isoniazid (5 mg/kg, up to 300 mg/day), ethambutol (15 mg/kg/day) and pyrazinamide (15–30 mg/kg, up to 2 gm/day) given for initial 2-months in the intensive phase. This is then followed by treatment with rifampicin and isoniazid during the continuation phase for next 4-months. The reported incidence of Mycobacterium tuberculosis resistance, to one or more first line drugs, is 10–14% in the U.K. and the U.S.A. (3).

It is now possible to detect drug resistance by molecular means or by using the light-producing enzyme Luciferase, the gene for which has been added to mycobacteriophage (4), instead of more time consuming culture and sensitivity techniques. Because the drug resistance patterns vary at a given time and place it is advisable to consult local specialists well-versed in this particular problem, as well as for their expertise in managing difficult cases and drug intolerance.

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Corticosteroids will continue to dominate the drug list for management of reactions in leprosy until an ideal substitute is found. The aim of all those dealing with leprosy and its complications is always to minimize steroid-induced side effects. Methotrexate, being the most widely used antimetabolite by dermatologists the world over, is a particularly important candidate since it is well established.

CASE HISTORY

A 60-yr-old man was referred to Karigiri hospital with fever and multiple skin nodules of 20 days duration. The patient was a diagnosed case of multibacillary (MB) Hansen’s disease and was released from treatment 4 months before upon completion of 12-month MB Multi-drug Therapy (MDT). He had no complaints of such attacks during treatment. He also complained of multiple joint pains along with pain along the medial border of left hand. On examination, the patient had multiple subcutaneous nodules of which a few had ulcerated over the back, face, and forearms. No skin patch was visible but there was diffuse skin infiltration with some areas of sparing. Both the ulnar nerves were thickened and the right ulnar had developed an abscess. Patient also had bilateral weakness of hands assessed by motor testing. The peripheral sensations were relatively preserved. On investigating, he had neutrophilia with toxic granules, slit skin smear was positive with a Bacillary Index (BI) of 3.75+ and Morphological Index of -0. Liver function tests and renal function tests were all within normal limits. ELISA for HIV was negative. Chest X-Ray was normal. No focus of infection could be found. Patient was diagnosed as a case of completely treated borderline lepromatous (BL) leprosy with severe Type 2 reaction and was started on systemic prednisolone.

Received for publication on 9 May 2004. Accepted for publication on 3 August 2004.

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CASE REPORTS

Methotrexate in Resistant ENL

Bikash R. Kar and Ravindra Babu

ABSTRACT

This is a report of a case of steroid resistant severe Type 2 reaction that was managed with methotrexate and prednisolone. Synergistic action of both the drugs in severe Type 2 reaction make them one of the preferred combinations in the absence of other agents such as thalidomide.

RÉSUMÉ

Cet article rapport un cas de réaction sévère de type 2, résistante aux corticostéroïdes, qui fut contrôlée par du méthotrexate et de la prednisolone. L’action synergique de ces deux médicaments dans les réactions sévères de type 2 en fait une des combinaisons préférées en l’absence d’autres composés tels que la thalidomide.

RESUMEN

Este es el reporte de un caso de reacción leprosa tipo 2 resistente a esteroides que fue tratado con metotrexato y prednisolona. La actividad sinérgica de estas dos drogas en el tratamiento de la reacción tipo 2 severa, hacen que esta combinación sea la preferida cuando no se cuenta con otros agentes como la talidomida.
40 mg/day. The abscess in the right ulnar nerve was managed with decompression and splinting. Erythema nodosum leprosum (ENL), as well as the features of toxicity, started subsiding within 4 days of initiation of steroid therapy, when the patient developed another crop of nodules along with fever. The dose of Prednisolone was increased to 50 mg/day. The patient was still developing a number of new lesions of ENL even at that dose. After 2 weeks, methotrexate was added to the regimen, as he was not responding to steroid monotherapy satisfactorily and thalidomide was not available. It was given at a dose of 5 mg 12 hourly for 3 doses every week as the gastric upset was supposed to be less compared to a single dose regimen. After 2 weeks, the dose of methotrexate was tapered by 2.5 mg/week and at present he is receiving a dose of 7.5 mg every week in a divided manner along with a daily dose of 20 mg of Prednisolone. The patient did not have any new ENL lesions after starting the additional methotrexate treatment.

**DISCUSSION**

Reactions are immune mediated complications seen in leprosy patients before, during, or after treatment with MDT. Pfaltzgraff, *et al.* (1) reported that almost half of the lepromatous cases and one quarter of borderline lepromatous cases experience ENL reaction. By definition, ENL is recurrent and self-limiting in the majority of cases. But some patients behave differently and are resistant to all modalities. Depending upon the severity of the ENL, various drugs are used ranging from non-steroidal anti-inflammatory agents in mild cases to corticosteroids and thalidomide (2) in severe cases. Thalidomide acts as a wonder drug in severe ENL (2) but it has its own limitations. Non-availability and high cost are the two major issues concerned with the routine use of the drug. The drug has to be given under supervision and is contraindicated in women of childbearing age in most of the countries. So practically speaking, corticosteroids are the mainstay in management of the condition. The WHO recommended dose is 40 mg/day (3) and majority respond to this regime. Prednisolone (2) acts by suppression of cell mediated immunity, inhibition of release of lysosomal enzymes and cytokines, decrease of fluid leakage at the site of inflammation, decrease in the response of neutrophils to chemotaxis, and inhibition of prostaglandin synthesis, etc. The drug has to be tapered over 4 months, though in some patients the dose has to be individualized. This is likely to lead to a lot of complications (3). So the use of this drug should be judicious and the search is always on to find an ideal steroid-sparing agent. Methotrexate is being used for the treatment of psoriasis since late 1950s (4) and remains one of the most commonly used antimetabolites in dermatology practice. Dermatologists have been using this drug routinely since then and the safety record is so far impressive though hepatic and bone marrow side effects are the major concerns (3). Low doses of methotrexate suppress division of mononuclear cells and inhibit their response to interleukin 2, suppress neutrophil and monocyte chemotaxis *in vitro* and *in vivo*, and depress Langerhans cell activity and leukotriene B4 synthesis by neutrophils (5, 9, 10, 11, 12, 13, 14), which also contribute to the manifestations of ENL. So a synergistic action of methotrexate to corticosteroids is expected in ENL cases; in other words, it would act like a steroid-sparing agent in those who are likely to be put on a high dose of prednisolone for a prolonged period. Taking into consideration the side effects of methotrexate, the risk benefit ratio should be carefully calculated and individualized while combining it with prednisolone for the steroid resistant cases. Double blind controlled trials are welcome in the near future.

**REFERENCES**


Deep vein thrombosis (DVT) is an important complication of several disorders and may sometimes occur spontaneously. An impaired fibrinolytic system (5), presence of lupus anti-coagulant activity (14) or increased levels of proinflammatory procoagulant cytokines (4) have all been linked to it. Cancer, surgery, fractures, puerperium, immobilization, paralysis, use of oral contraceptives, and anti-phospholipid syndrome (10), deficiencies of anti-thrombin, protein C, protein S and factor V Leiden, a mutation in coagulation factor, dysfibrogenemia and hyperhomocysteinemia (5) may be responsible for DVT. It is not a known complication of leprosy or lepra reactions and has not been reported in erythema nodosum leprosum (ENL) patients receiving thalidomide. Thalidomide, a sedative drug, has been in use for management of recurrent/severe ENL due to its anti-inflammatory properties which are attributed to inhibition of inflammatory cytokines, tumor necrosis factor alpha (TNF-α) (6), vascular endothelial derived growth factor (VEGF) and basic fibroblast growth factor (b FGF) in RNA processing (2, 6). It is also used in malignancies such as multiple myeloma (MM) and renal cell carcinoma (RCC) (2, 13).

A higher incidence of deep vein thrombosis has been observed in cancer patients receiving thalidomide (12), and the incidence increases several folds when thalidomide is combined with other chemo-therapeutic agents (8, 15).

We report here a case of difficult to control recurrent ENL on treatment with oral prednisolone and thalidomide, who developed DVT when dexamethasone-cyclophosphamide (DCP) pulse was given additionally.
CASE REPORT

A 37-yr-old female patient was hospitalized with difficult to control severe and recurrent ENL. She was being treated at a peripheral health care center with multibacillary multidrug therapy (MB-MDT) for the last one year and prednisolone 40 mg/day for the past three months without adequate control of ENL. She had already developed iatrogenic cushingoid syndrome.

She had high fever, myalgia, arthralgia, recurrent crops of tender and necrotic ENL lesions, and ulnar nerve neuritis. Initially, her prednisolone dose was increased to 60 mg/day and then gradually tapered to 40 mg/day after the control of symptoms. As there was severe exacerbation of the symptoms after reduction of the prednisolone dose to <50 mg/day on three occasions; thalidomide was added in the dose of 100 mg twice daily. There was no regression of symptoms in three weeks, therefore, the dose of thalidomide was increased to 100 mg thrice daily. There was no noticeable improvement of symptoms with this regimen even after two weeks. At this point, based on our previous experience (7), it was decided to add dexamethasone-cyclophosphamide (DC) pulse. The regimen consists of slow intra-venous infusion (in over 2 hr) of 100 mg of dexamethasone in 500 ml of 5% glucose on three consecutive days with 500 mg of cyclophosphamide added to this on second day. This regimen is repeated after every 28 days. Cyclophosphamide 50 mg/day is given in the intervening period. The daily dose of oral prednisolone was reduced by 10 mg after each DC pulse. There was a noticeable improvement in the symptoms after the addition of DC pulse.

Five days after the second DC pulse, patient developed swelling and severe pain in her left leg. Doppler ultrasonography revealed massive adherent thrombosis in her left external iliac vein extending to the common iliac vein. Various laboratory parameters including the platelet counts were within normal limits. With the development of this complication, thalidomide was discontinued immediately and the patient was managed with subcutaneous low molecular weight heparin 3000 anti-Xa units twice daily for initial seven days and subsequently oral warfarin. The dosage of warfarin was adjusted so as to maintain the International Normalized Ratio (I.N.R.) between 2 to 3. Patient’s leg pain and swelling improved considerably and she became symptom free in about a month. She is currently on warfarin 2 mg on alternate days. After seven DC pulses, she is free of her recurrent ENL attacks and we have also been able to bring down her prednisolone dose. At present she is on MB-MDT, cyclophosphamide 50 mg/day and prednisolone 10 mg on alternate days. No other side effects of this regimen have been recorded and patient was doing well in a follow-up of about 9 months.

DISCUSSION

Chronic recurrent ENL is a serious and troublesome complication of leprosy and is often difficult to control. The clinical features of ENL are attributed to increased levels of TNF-α and interferon-γ (11). Thalidomide reverses this phenomenon and also induces reduction in neutrophils, CD4+ T cells, TNF-α cells, MHC class-II antigens and ICAM-1 on epidermal keratinocytes and thus effectively controls ENL (11). However its routine use is severely restricted due to its serious toxicities like phocomelia, neuropathies, hyperkalemia and deep vein thrombosis (3, 9).

Acute DVT is a serious and potentially fatal disorder that commonly complicates the course of many diseases in bed ridden patients. It has also been observed that there is unexpectedly higher incidence of DVT when thalidomide is used along with doxorubicin, gemcitabine, 5-Fluorouracil, darbepoietin-α, or dexamethasone-cyclophosphamide therapy (6). Evidently, these observations have been made in patients being treated for metastatic cell carcinoma, myelodysplastic syndrome, or multiple myeloma. To the best of our knowledge no such association has been reported in ENL reactions (when treated with thalidomide alone).

Thalidomide, chemotherapy and glucocorticoids induce apoptosis in tumor cells in vitro and these apoptotic cells might be thrombogenic because of increased activation of tissue factors in the plasma membrane of tumor cells (1), has been shown to inhibit endothelial cell poliferation because of free radical mediated oxidative DNA damage which impairs endothelial cell
function (8). It also induces Th-1 cellular response leading to increased secretion of interferon-γ and interleukin-2 (11). Thalidomide is also a potent angiogenesis inhibitor. Thromboembolic events have been reported to occur at a mean of about two months after the start of thalidomide treatment (9). In our patient, DVT developed after eight weeks of starting thalidomide therapy and five weeks of DC pulse therapy.

In such a situation, the question remains that whether the increased risk of DVT is due to thalidomide alone or due to an interaction between thalidomide and other chemotherapeutic agents. Thalidomide, in spite of its limitations, is the drug of choice in severe ENL, and will probably find new usages in future. The clinicians need be vigilant about potential occurrence of thrombotic complications in these patients especially when glucocorticoids or other chemotherapeutic agents are being used concomitantly.

REFERENCES

CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.

Using the Blink Reflex as Measured by Electromyogram to Assess Cranial Nerve Involvement in People Affected by Leprosy

Hazir Ulvi, Remzi Yigiter, Tahir Yoldas, Recep Aygul, Asuman Orhan Varoglu, and Orhan Deniz

ABSTRACT

Damage to the peripheral nervous system is particularly frequent in leprosy patients. Trigeminal and facial nerves are among the most commonly affected. The aim of our study was to evaluate the efficacy of the blink reflex as a method for diagnosis of cranial nerve involvement in people affected by leprosy. We studied 37 affected people (mean age: 38 yrs, 20 female and 17 male) and 35 age-matched healthy subjects (mean age: 34 yrs, 20 female and 15 male). Blink reflexes were obtained after unilateral electrical stimulation of the supraorbital nerve for quantitative analysis of 3 responses, early ipsilateral phasic component (R1), late ipsilateral tonic component (R2i), and late contralateral tonic component (R2c). Nerve conduction parameters were studied in all subjects.

The latencies of both the ipsilateral early phasic component (R1) and bilateral late tonic components (R2i and R2c) in people affected by leprosy were significantly prolonged compared with the controls. Out of 37 people affected by leprosy, 22 (59%) showed abnormalities R1 latency, 28 (75%) R2i latency and 31 (83%) R2c latency. No correlation was observed between prolonged latencies and duration of the disease. We conclude that blink reflex testing, which can be easily and rapidly performed in an EMG laboratory using standard equipment, can provide useful and objective information for the diagnosis of cranial nerve lesions and for the determination of the degree thereof.

RÉSUMÉ

Les atteintes du système nerveux périphérique sont particulièrement fréquentes chez les patients lépreux. Les nerfs trijumeaux et faciaux sont parmi les nerfs les plus souvent affectés. Le but de cette étude était d’évaluer l’efficacité du réflexe de clignement comme méthode de diagnostic d’atteinte des nerfs crâniens chez les personnes affectées par la lèpre. Nous avons étudié 37 personnes affectées (âge moyen : 38 ans, 20 femmes et 17 hommes)
TO THE EDITOR:

Leprosy remains an important health problem worldwide. Leprosy was once widely distributed in Europe and Asia but now occurs mainly in resource-poor countries in tropical and warm temperate regions (1). According to the World Health Organization (WHO), at the beginning of 2003, the number of leprosy patients in the world was around 534,000, as reported by 110 countries. About 621,000 new cases were detected during 2002 (2). It is a very serious and mutilating disease in many parts of the world and diagnosis and therapy is the most important strategy for its control (12). All patients with leprosy have some degree of nerve involvement, making leprous neuritis undoubtedly the most common cause of treatable neuropathy in the world. Deformities from involvement of facial structures, eyes, nerves, bone, and skin can result in stigmatization and social ostracism. The diagnosis of leprosy is often missed by physicians in the United States, leading to delay of treatment during which progressive neuropathy, visual loss and deformity may occur (13). Diagnostic delay in leprosy can have serious neurological consequences for the patient. Lockwood, et al. (8) demonstrated that the median time from symptom onset to diagnosis was 1.8 yrs (0.2 to 15.2) and delayed in diagnosis occurred in 82% of cases in the United Kingdom. They also demonstrated that misdiagnosis as other dermatological and neurological conditions were important causes of delay 68% of patients in the UK were found to have nerve damage resulting in disability. Increased awareness among general practitioners and hospital specialists would lead to more rapid diagnosis, thus minimizing damage.

RESUMEN

El daño al sistema nervioso periférico es particularmente frecuente entre los pacientes con lepra. Los nervios trigémino y facial están entre los más frecuentemente afectados. El objetivo del estudio fue evaluar la eficacia del parpadeo reflejo como un método de diagnóstico de afección del nervio craneal en pacientes con lepra. Se estudiaron 37 pacientes (edad promedio: 38 años, 20 mujeres y 15 hombres) y 35 sujetos sanos apareados por edad (edad promedio: 34 años, 20 mujeres y 15 hombres). Los reflejos de parpadeo se indujeron por estimulación eléctrica unilateral del nervio supraorbital y se evaluaron cuantitativamente 3 respuestas: el componente fásico ipsilateral temprano (R1), el componente tónico ipsilateral tardío (R2i), y el componente tónico contralateral (R2c). En todos los sujetos también se estudiaron los parámetros de conducción nerviosa. En comparación con los controles sanos, las latencias tanto del componente fásico ipsilateral temprano (R1) como de los componentes tónicos bilaterales tardíos (R2i y R2c) en los pacientes con lepra estuvieron significativamente prolongados. De 37 personas afectadas por la lepra, 22 (59%) mostraron anormalidades en la latencia R1, 28 (75%) en la latencia R2i, y 31 (83%) en la latencia R2c. No se observó correlación entre las latencias prolongadas y la duración de la enfermedad. Concluimos que la prueba de reflejos de parpadeo, la cual puede hacerse fácil- y rápidamente en un laboratorio de EMG usando equipo estándar, puede proporcionar información útil y objetiva para el diagnóstico de las lesiones en el nervio craneal y para determinar su grado de afección.
and disability. When detected and treated early, primary impairments may be reversible (21). For this reason, early recognition of leprosy is very important.

Trigeminal and facial nerves are among the most commonly affected in leprosy patients (11). Electrophysiological studies such as the blink reflex have been shown to be an effective method for revealing subclinical involvement of cranial nerves in generalised neuropathies (4, 5, 9, 11). The aim of our study was to evaluate the efficacy of the blink reflex as a method for diagnosis of cranial nerve involvement in people affected by leprosy.

METHODS

We studied 37 patients who had been treated for lepromatous leprosy (LL) with a mean age of 38 ± 17 yrs (range 23 yrs to 62 yrs; 20 female and 17 male), and 35 age-matched healthy volunteer subjects (control) with a mean age of 34 ± 12 yrs (range 24 yrs to 48 yrs; 20 female and 15 male). The demographic characteristics of patients and control subjects are presented in Table 1. Patients were hospitalized in Elazıg Leprosy Hospital for treatment and rehabilitation. The patients involved in this study had completed treatment and were cured of leprosy. The period between diagnosis of leprosy and study was 16.73 yrs. The patients and controls were carefully examined. All subjects in the study had a negative history and negative physical examination for central nervous system disease.

Exclusion Criteria:

• alcohol abuse
• cigarette consumption of over 10 cigarettes/day
• the presence of disease of the peripheral nervous system not related to leprosy
• tuberculoid and borderline leprosy (according to the Ridley-Jopling classification) (23)

Informed consent was obtained from all patients and controls before the investigations were carried out. Routine blood analysis was performed.

All subjects were studied using electromyogram (EMG) equipment (KEY-POINT, DANTEC, DENMARK). Following the standardized protocol proposed by Kimura, et al. (6) the supraorbital nerve in the supraorbital foramen was stimulated and the evoked responses from both orbicularis oculi muscles were recorded. Subjects were awake, in dorsal decubitus position, with room temperatures between 22 and 27°C, in a semi-darkened room. Surface platinum disc electrodes of 0.5 cm diameter were positioned as follows: channel 1, active electrode G1 was placed on the belly of the left orbicularis oculi muscle, 1 cm below the left lateral epicanthal point; reference electrode G2 was placed on the lateral surface of the nose; channel 2 was symmetrically positioned in relation to the channel 1 electrodes, on the right side. Fil-

<table>
<thead>
<tr>
<th>Sex</th>
<th>Patients</th>
<th>Control group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Age (yrs)</td>
</tr>
<tr>
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</tr>
<tr>
<td>Male</td>
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</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>38</td>
</tr>
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Correspondence

Table 2. Mean values of some parameters in the leprosy patients and the control group.

<table>
<thead>
<tr>
<th></th>
<th>Leprosy patients</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left eye R1 mean latency (msec)</td>
<td>12.68 ± 3.43</td>
<td>11.28 ± 2.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Right eye R1 mean latency (msec)</td>
<td>13.01 ± 2.07</td>
<td>11.14 ± 2.18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left eye R2i mean latency (msec)</td>
<td>37.4 ± 6.55</td>
<td>32.51 ± 6.65</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Right eye R2i mean latency (msec)</td>
<td>38.25 ± 4.72</td>
<td>30.23 ± 3.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left eye R2c mean latency (msec)</td>
<td>38.01 ± 9.76</td>
<td>32.91 ± 4.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Right eye R2c mean latency (msec)</td>
<td>39.76 ± 3.48</td>
<td>31.67 ± 5.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Median nerve motor distal latency (msec)</td>
<td>4.1 ± 0.58</td>
<td>3.4 ± 0.56</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ulnar nerve motor distal latency (msec)</td>
<td>4.6 ± 0.84</td>
<td>3.2 ± 0.54</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

ter band-pass was set to 20–3000 Hz, sensitivity to 200 µV/cm, and the sweep velocity was 10 m/sec per division. Stimulation was performed with the cathode over the supraorbital foramen, with single stimuli on each side, consisting of square-wave pulses of 0.2 m/sec duration and 25 mA intensity. The ground electrode was positioned comfortably around the neck. Two recordings were obtained from both sides for each subject, with ten seconds or more between stimuli. Blink reflexes were obtained after unilateral electric stimulation of the supraorbital nerve for quantitative analysis of 3 responses, early ipsilateral phasic component (R1), late ipsilateral tonic component (R2i) and late contralateral tonic component (R2c). Latency was measured from the stimulus onset to the shortest initial deflection of the height R1 or R2 components. Amplitude was not important. Examples of blink reflex in a control subject and a patient are presented in Fig. 1.

Statistical methods. Data are expressed as mean ± standard deviation (mean ± S.D.). Differences between the means of groups were determined using the unpaired t-test. The correlation of two parameters was tested using a linear regression analysis. Values of p <0.05 were accepted as statistically significant.

RESULTS

The data from each group studied are summarized in Table 2. The latencies of both the ipsilateral early phasic component (R1) and bilateral late tonic components (R2i and R2c) in people affected by leprosy were significantly prolonged compared with the controls (Fig. 1). In general, abnormal R1 latency was less than that of R2. Out of 37 people affected by leprosy, 22 (59%) showed abnormalities R1 latency, 28 (75%) R2i latency and 31 (83%) R2c latency. No correlation was observed between prolonged latencies and the duration of disease.

DISCUSSION

Trigeminal and facial nerves are among the most commonly affected in people affected by leprosy (11). Diagnostic delay in leprosy can have serious neurological consequences for the patient. The diagnosis of leprosy is often missed by physicians, leading to delay of treatment during which progressive neuropathy, visual loss, and deformity may occur (8, 13). It is a very serious, mutilating and stigmatizing disease in many parts of the world and early diagnosis and therapy is the most important strategy for its control (3, 8, 13). There is evidence that nerve damage in leprosy occurs before clinical manifestations become apparent (16, 17, 20). When the clinical signs are apparent, extensive and often-permanent nerve damage has already taken place. A method to detect asymptomatic neuropathy of trigeminal and facial nerves electrophysiologically could be valuable to identify patients at high risk for symptomatic cranial neuropathy. One opportunity for this is the measurement of damage to trigeminal and facial nerves using the blink reflex in standard EMG equipment.

The blink reflex is a very practical, reproducible electrical response, which can be used in comparative clinical studies and experimental models (6, 18). It is an electrically induced glabellar response that has long been used in clinical neurology and is known to be a polisinaptic reflex with an afferent arc through sensory fibers of the trigeminal nerve and with an efferent arc through the motor fibers of the facial nerve (6, 7, 14). It has been most useful in the evaluation of lesions affecting the trigeminal nerve; however, it has been less useful in the evaluation of lesions affecting the facial nerve.
nerve and facial nerve (1, 2). For the detection of a lesion in the first division of trigeminal nerve, the blink reflex is the only physiological test available at this time. The classic findings indicative of such a lesion are an afferent defect, a prolonged latency of R1, R2i and R2c (2). In facial nerve lesions, there is a delay in the reflex latency only on the affected side, regardless of the side of the stimulation (6–9).

In our study, latency of R1, R2i and R2c were significantly prolonged in the people affected by leprosy compared to the control subjects, similar to the findings in previous studies (5, 11). These results, together with previous studies, demonstrate that some degree of cranial neuropathy may be present in the people affected by leprosy with neuropathy. The blink reflex study may indicate an effective diagnosis of cranial nerve involvement.

Shetty, et al. (17) reported that subclinical neuropathy may take place before clinical manifestations become apparent. Indeed, histopathological and immunocytochemical studies have demonstrated that nerve damage progresses from small unmyelinated to small myelinated and finally to large myelinated fibers (15). The authors suggest that this electrophysiological study may well indicate subclinical neuropathy in these patients, since a significantly prolonged latency was not seen in the normal control subjects.

Ramachandran, et al. (10) reported an association between the severity of autonomic neuropathy and a longer duration of leprosy. In this study, no significant correlation between duration of leprosy and positive test results was found. This was probably due to a longer duration of the effects of leprosy after treatment in our patients. The period between diagnosis of leprosy and study was 16.73 yrs.

In conclusion, we have observed markedly abnormal patterns of blink reflex in leprosy. The use of blink reflex testing which can be easily and rapidly performed in an EMG laboratory using standard equipment can provide useful, objective information for diagnosis and in determining the degree of cranial nerve lesions. However, future electrophysiological trials in active leprosy will provide further information for the use of this test in the early diagnosis of cranial nerve involvement in leprosy patients.

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Regarding Classification of Leprosy

TO THE EDITOR:

I have just read your editorial on classification of leprosy in the June issue of the JOURNAL. It is a very thought-provoking and stimulating article.

Unfortunately it is too true that we are not asking such fundamental questions very often. It becomes much easier to simplify everything in life and then imagine that the simplified representations are the reality.

I do agree with you that simplifying the classification system has been an invaluable tool to reach millions of untreated individuals and to lower prevalence, and it still remains a valid tool for public health. However it should not be confused with our endeavor to understand a disease through scientific research.

—Dr. Sunil Deepak, Director

Medical Support Department
AIFO - Italy

Epidemiology of Leprosy

TO THE EDITOR:

I read with great interest the article titled “Epidemiological Characteristics of Leprosy Reactions: 15 years experience from North India,” Int. J. Lepr. Other Mycobact. Dis. 72 (2004) 125–133. Prof. Bhushan Kumar and his co-authors have to be complimented on a painstaking analysis of 2600 patients with leprosy reactions attending their reputed “tertiary care institute in Northern India, which is a low endemic area for leprosy.” This is indeed a very useful clinical contribution.

However, I wonder whether it is an “epidemiological study” (as the title implies) based on a specific population from which the sample of patients is derived. As far as I know “Epidemiology” is defined as the study of the distribution and determinants of disease in human population. Whereas the basis of clinical research is the observation on individual patients, epidemiology requires observation of communities of people among whom disease occurs. The word epidemiology means something about people (EPI = upon; DEMOS = people).

While factors like onset, risk factors like age, sex etc. which also form important parameters of epidemiology are well described in the article, the occurrence of reactions and incidence over a period of time in specific communities or population groups is not available. This information will be necessary for planning management of reactions under field conditions. The title of the paper may be a misnomer.

I would invite comments from the authors or any epidemiologist on these points.

—Dr. R. Ganapati

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Mumbai – 400 022, India
THE AUTHOR’S REPLY:

We thank Dr. Ganapati for his interest, appreciation, and comments regarding our article “Epidemiological Characteristics of Leprosy Reactions: 15 years experience from North India,” which appeared in Vol. 72 (2004) of THE INTERNATIONAL JOURNAL OF LEPROSY AND OTHER MYCOBACTERIAL DISEASES, pp. 125–133.

The definition of “epidemiology” in its true sense as mentioned by Dr. Ganapati is well known and accepted by all the health care professionals. However, we used the word “epidemiological characteristics” to describe various parameters/variables of reactions in relation to our large group of leprosy patients.

The risk factors and incidence over a period of time is discussed in relation to pauci- and multibacillary disease. It was beyond the scope of this hospital-based analysis to interpret results with respect to “communities” and “population groups.”

Our results do provide important inputs for planning management of reactions both under institutional and field conditions, however being a hospital based study, both the incidence and severity of leprosy reactions may not be totally comparable to the situation in field. We have also given the data from the field studies vis-à-vis the hospital based figures for purposes of completeness and to help the readers/epidemiologists to draw their own conclusions.

We are thankful to Dr. Ganapati and your journal for giving us the opportunity to put across our point of view about the basic and the practical usage of a term.

—Bhushan Kumar M.D., MNAMS, Sunil Dogra M.D., DNB MNAMS, Inderjeet Kaur M.D., MNAMS, Department of Dermatology, Venereology & Leprology Postgraduate Institute of Medical Education and Research, Chandigarh, India
CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.

Using the Blink Reflex as Measured by Electromyogram to Assess Cranial Nerve Involvement in People Affected by Leprosy

Hazir Ulvi, Remzi Yigiter, Tahir Yoldas, Recep Aygul, Asuman Orhan Varoglu, and Orhan Deniz

ABSTRACT

Damage to the peripheral nervous system is particularly frequent in leprosy patients. Trigeminal and facial nerves are among the most commonly affected. The aim of our study was to evaluate the efficacy of the blink reflex as a method for diagnosis of cranial nerve involvement in people affected by leprosy. We studied 37 affected people (mean age: 38 yrs, 20 female and 17 male) and 35 age-matched healthy subjects (mean age: 34 yrs, 20 female and 15 male). Blink reflexes were obtained after unilateral electrical stimulation of the supraorbital nerve for quantitative analysis of 3 responses, early ipsilateral phasic component (R1), late ipsilateral tonic component (R2i), and late contralateral tonic component (R2c). Nerve conduction parameters were studied in all subjects.

The latencies of both the ipsilateral early phasic component (R1) and bilateral late tonic components (R2i and R2c) in people affected by leprosy were significantly prolonged compared with the controls. Out of 37 people affected by leprosy, 22 (59%) showed abnormalities R1 latency, 28 (75%) R2i latency and 31 (83%) R2c latency. No correlation was observed between prolonged latencies and duration of the disease. We conclude that blink reflex testing, which can be easily and rapidly performed in an EMG laboratory using standard equipment, can provide useful and objective information for the diagnosis of cranial nerve lesions and for the determination of the degree thereof.

RÉSUMÉ

Les atteintes du système nerveux périphérique sont particulièrement fréquentes chez les patients lépreux. Les nerfs trijumeaux et faciaux sont parmi les nerfs les plus souvent affectés. Le but de cette étude était d’évaluer l’efficacité du réflexe de clignement comme méthode de diagnostic d’atteinte des nerfs crâniens chez les personnes affectées par la lèpre. Nous avons étudié 37 personnes affectées (âge moyen : 38 ans, 20 femmes et 17 hommes)

1Received for publication on 13 April 2004. Accepted for publication 2 July 2004.
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TO THE EDITOR:

Leprosy remains an important health problem worldwide. Leprosy was once widely distributed in Europe and Asia but now occurs mainly in resource-poor countries in tropical and warm temperate regions (3). According to the World Health Organization (WHO), at the beginning of 2003, the number of leprosy patients in the world was around 534,000, as reported by 110 countries. About 621,000 new cases were detected during 2002 (22). It is a very serious and mutilating disease in many parts of the world and diagnosis and therapy is the most important strategy for its control (12). All patients with leprosy have some degree of nerve involvement, making leprous neuritis undoubtedly the most common cause of treatable neuropathy in the world. Deformities from involvement of facial structures, eyes, nerves, bone, and skin can result in stigmatization and social ostracism. The diagnosis of leprosy is often missed by physicians in the United States, leading to delay of treatment during which progressive neuropathy, visual loss and deformity may occur (13). Diagnostic delay in leprosy can have serious neurological consequences for the patient. Lockwood, et al. (8) demonstrated that the median time from symptom onset to diagnosis was 1.8 yrs (0.2 to 15.2) and delayed in diagnosis occurred in 82% of cases in the United Kingdom. They also demonstrated that misdiagnosis as other dermatological and neurological conditions were important causes of delay 68% of patients in the UK were found to have nerve damage resulting in disability. Increased awareness among general practitioners and hospital specialists would lead to more rapid diagnosis, thus minimizing damage.

RESUMEN

El daño al sistema nervioso periférico es particularmente frecuente entre los pacientes con lepra. Los nervios trigémino y facial están entre los más frecuentemente afectados. El objetivo del estudio fue evaluar la eficacia del parpadeo reflejo como un método de diagnóstico de afección del nervio craneal en pacientes con lepra. Se estudiaron 37 pacientes (edad promedio: 38 años, 20 mujeres y 17 hombres) y 35 sujetos sanos apareados por edad (edad promedio 34 años, 20 mujeres y 15 hombres). Los reflejos de parpadeo se indujeron por estimulación eléctrica unilateral del nervio supraorbital y se evaluaron cuantitativamente 3 respuestas: el componente fásico ipsilateral temprano (R1), el componente tónico ipsilateral tardío (R2i), y el componente tónico contralateral (R2c). En todos los sujetos también se estudiaron los parámetros de conducción nerviosa. En comparación con los controles sanos, las latencias tanto del componente fásico ipsilateral temprano (R1) como de los componentes tónicos bilaterales tardíos (R2i y R2c) en los pacientes con lepra estuvieron significativamente prolongados. De 37 personas afectadas por la lepra, 22 (59%) mostraron anormalidades en la latencia R1, 28 (75%) en la latencia R2i, y 31 (83%) en la latencia R2c. No se observó correlación entre las latencias prolongadas y la duración de la enfermedad. Concluimos que la prueba de reflejos de parpadeo, la cual puede hacerse fácil- y rápidamente en un laboratorio de electromiografía usando equipo estándar, puede proporcionar información útil y objetiva para el diagnóstico de las lesiones en el nervio craneal y para determinar su grado de afección.
and disability. When detected and treated early, primary impairments may be reversible \((21)\). For this reason, early recognition of leprosy is very important.

Trigeminal and facial nerves are among the most commonly affected in leprosy patients \((11)\). Electrophysiological studies such as the blink reflex have been shown to be an effective method for revealing subclinical involvement of cranial nerves in generalised neuropathies \((4,5,9,11)\). The aim of our study was to evaluate the efficacy of the blink reflex as a method for diagnosis of cranial nerve involvement in people affected by leprosy.

**METHODS**

We studied 37 patients who had been treated for lepromatous leprosy (LL) with a mean age of \(38 \pm 17\) yrs (range 23 yrs to 62 yrs; 20 female and 17 male), and 35 age-matched healthy volunteer subjects (control) with a mean age of \(34 \pm 12\) yrs (range 24 yrs to 48 yrs; 20 female and 15 male). The demographic characteristics of patients and control subjects are presented in Table 1. Patients were hospitalized in Elazıg Leprosy Hospital for treatment and rehabilitation. The patients involved in this study had completed treatment and were cured of leprosy. The period between diagnosis of leprosy and study was \(16.73\) yrs. The patients and controls were carefully examined. All subjects in the study had a negative history and negative physical examination for central nervous system disease.

**Exclusion Criteria:**

- alcohol abuse
- cigarette consumption of over 10 cigarettes/day
- the presence of disease of the peripheral nervous system not related to leprosy
- tuberculoid and borderline leprosy (according to the Ridley-Jopling classification) \((23)\)

![The Figure. Examples of blink reflex in an adult control subject (upper) and a patient of similar age with leprosy (lower).](image)

Informed consent was obtained from all patients and controls before the investigations were carried out. Routine blood analysis was performed.

All subjects were studied using electromyogram (EMG) equipment (KEYPOINT, DANTEC, DENMARK). Following the standardized protocol proposed by Kimura, et al. \((6)\) the supraorbital nerve in the supraorbital foramen was stimulated and the evoked responses from both orbicularis oculi muscles were recorded. Subjects were awake, in dorsal decubitus position, with room temperatures between 22 and \(27^\circ\)C, in a semi-darkened room. Surface platinum disc electrodes of 0.5 cm diameter were positioned as follows: channel 1, active electrode G1 was placed on the belly of the left orbicularis oculi muscle, 1 cm below the left lateral epicanthal point; reference electrode G2 was placed on the lateral surface of the nose; channel 2 was symmetrically positioned in relation to the channel 1 electrodes, on the right side. Fil-

<table>
<thead>
<tr>
<th>Sex</th>
<th>Patients</th>
<th>Control group</th>
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<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Age (yrs)</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>38</td>
</tr>
</tbody>
</table>
Correspondence

ter band-pass was set to 20–3000 Hz, sensitivity to 200 µV/cm, and the sweep velocity was 10 msec per division. Stimulation was performed with the cathode over the supraorbital foramen, with single stimuli on each side, consisting of square-wave pulses of 0.2 msec duration and 25 mA intensity. The ground electrode was positioned comfortably around the neck. Two recordings were obtained from both sides for each subject, with ten seconds or more between stimuli. Blink reflexes were obtained after unilateral electric stimulation of the supraorbital nerve for quantitative analysis of 3 responses, early ipsilateral phasic component (R1), late ipsilateral tonic component (R2i) and late contralateral tonic component (R2c). Latency was measured from the stimulus onset to the shortest initial deflection of the height R1 or R2 components. Amplitude was not important. Examples of blink reflex in a control subject and a patient are presented in Fig. 1.

Statistical methods. Data are expressed as mean ± standard deviation (mean ± S.D.). Differences between the means of groups were determined using the unpaired t-test. The correlation of two parameters was tested using a linear regression analysis. Values of p <0.05 were accepted as statistically significant.

RESULTS

The data from each group studied are summarized in Table 2. The latencies of both the ipsilateral early phasic component (R1) and bilateral late tonic components (R2i and R2c) in people affected by leprosy were significantly prolonged compared with the controls (Fig. 1). In general, abnormal R1 latency was less than that of R2. Out of 37 people affected by leprosy, 22 (59%) showed abnormalities R1 latency, 28 (75%) R2i latency and 31 (83%) R2c latency. No correlation was observed between prolonged latencies and the duration of disease.

DISCUSSION

Trigeminal and facial nerves are among the most commonly affected in people affected by leprosy (11). Diagnostic delay in leprosy can have serious neurological consequences for the patient. The diagnosis of leprosy is often missed by physicians, leading to delay of treatment during which progressive neuropathy, visual loss, and deformity may occur (8, 13). It is a very serious, mutilating and stigmatizing disease in many parts of the world and early diagnosis and therapy is the most important strategy for its control (3, 8, 13). There is evidence that nerve damage in leprosy occurs before clinical manifestations become apparent (16, 17, 20). When the clinical signs are apparent, extensive and often-permanent nerve damage has already taken place. A method to detect asymptomatic neuropathy of trigeminal and facial nerves electrophysiologically could be valuable to identify patients at high risk for symptomatic cranial neuropathy. One opportunity for this is the measurement of damage to trigeminal and facial nerves using the blink reflex in standard EMG equipment.

The blink reflex is a very practical, reproducible electrical response, which can be used in comparative clinical studies and experimental models (6, 18). It is an electrically induced glabellar response that has long been used in clinical neurology and is known to be a polysynaptic reflex with an afferent arc through sensory fibers of the trigeminal nerve and with an efferent arc through the motor fibers of the facial nerve (6, 7, 14). It has been most useful in the evaluation of lesions affecting the trigeminal nerves.

<table>
<thead>
<tr>
<th></th>
<th>Leprosy patients</th>
<th>Control group</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Left eye R1 mean latency (msec)</td>
<td>12.68 ± 3.43</td>
<td>11.28 ± 2.32</td>
<td>&lt;0.05</td>
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<tr>
<td>Right eye R1 mean latency (msec)</td>
<td>13.01 ± 2.07</td>
<td>11.14 ± 2.18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left eye R2i mean latency (msec)</td>
<td>37.4 ± 6.55</td>
<td>32.51 ± 6.65</td>
<td>&lt;0.05</td>
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<td>Right eye R2i mean latency (msec)</td>
<td>38.25 ± 4.72</td>
<td>30.23 ± 3.6</td>
<td>&lt;0.05</td>
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<tr>
<td>Left eye R2c mean latency (msec)</td>
<td>38.01 ± 9.76</td>
<td>32.91 ± 4.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Right eye R2c mean latency (msec)</td>
<td>39.76 ± 3.48</td>
<td>31.67 ± 5.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Median nerve motor distal latency (msec)</td>
<td>4.1 ± 0.58</td>
<td>3.4 ± 0.56</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ulnar nerve motor distal latency (msec)</td>
<td>4.6 ± 0.84</td>
<td>3.2 ± 0.54</td>
<td>&lt;0.05</td>
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TABLE 2. Mean values of some parameters in the leprosy patients and the control group.
nerve and facial nerve (1, 2). For the detection of a lesion in the first division of trigeminal nerve, the blink reflex is the only physiological test available at this time. The classic findings indicative of such a lesion are an afferent defect, a prolonged latency of R1, R2i and R2c (3). In facial nerve lesions, there is a delay in the reflex latency only on the affected side, regardless of the side of the stimulation (6–9).

In our study, latency of R1, R2i and R2c were significantly prolonged in the people affected by leprosy compared to the control subjects, similar to the findings in previous studies (5-11). These results, together with previous studies, demonstrate that some degree of cranial neuropathy may be present in the people affected by leprosy with neuropathy. The blink reflex study may indicate an effective diagnosis of cranial nerve involvement.

Shetty, et al. (17) reported that subclinical neuropathy may take place before clinical manifestations become apparent. Indeed, histopathological and immunocytochemical studies have demonstrated that nerve damage progresses from small unmyelinated to small myelinated and finally to large myelinated fibers (15). The authors suggest that this electrophysiological study may well indicate subclinical neuropathy in these patients, since a significantly prolonged latency was not seen in the normal control subjects.

Ramachandran, et al. (10) reported an association between the severity of autonomic neuropathy and a longer duration of leprosy. In this study, no significant correlation between duration of leprosy and positive test results was found. This was probably due to a longer duration of the effects of leprosy after treatment in our patients. The period between diagnosis of leprosy and study was 16.73 yrs.

In conclusion, we have observed markedly abnormal patterns of blink reflex in leprosy. The use of blink reflex testing which can be easily and rapidly performed in an EMG laboratory using standard equipment can provide useful, objective information for diagnosis and in determining the degree of cranial nerve lesions. However, future electrophysiological trials in active leprosy will provide further information for the use of this test in the early diagnosis of cranial nerve involvement in leprosy patients.

REFERENCES
17. SHETTY, V. P., MEHTA, N. H., IRANI, P. F., and ANTIA, N. H. Study of evaluation of nerve damage in


Regarding Classification of Leprosy

TO THE EDITOR:

I have just read your editorial on classification of leprosy in the June issue of the JOURNAL. It is a very thought-provoking and stimulating article. Unfortunately it is too true that we are not asking such fundamental questions very often. It becomes much easier to simplify everything in life and then imagine that the simplified representations are the reality.

I do agree with you that simplifying the classification system has been an invaluable tool to reach millions of untreated individuals and to lower prevalence, and it still remains a valid tool for public health. However it should not be confused with our endeavor to understand a disease through scientific research.

—Dr. Sunil Deepak, Director Medical Support Department AIFO - Italy

Epidemiology of Leprosy

TO THE EDITOR:

I read with great interest the article titled “Epidemiological Characteristics of Leprosy Reactions: 15 years experience from North India,” Int. J. Lepr. Other Mycobact. Dis. 72 (2004) 125–133. Prof. Bhushan Kumar and his co-authors have to be complimented on a painstaking analysis of 2600 patients with leprosy reactions attending their reputed “tertiary care institute in Northern India, which is a low endemic area for leprosy.” This is indeed a very useful clinical contribution.

However, I wonder whether it is an “epidemiological study” (as the title implies) based on a specific population from which the sample of patients is derived. As far as I know “Epidemiology” is defined as the study of the distribution and determinants of disease in human population. Whereas the basis of clinical research is the observation on individual patients, epidemiology requires observation of communities of people among whom disease occurs. The word epidemiology means something about people (EPI = upon; DEMOS = people).

While factors like onset, risk factors like age, sex etc. which also form important parameters of epidemiology are well described in the article, the occurrence of reactions and incidence over a period of time in specific communities or population groups is not available. This information will be necessary for planning management of reactions under field conditions. The title of the paper may be a misnomer.

I would invite comments from the authors or any epidemiologist on these points.

—Dr. R. Ganapati

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The definition of “epidemiology” in its true sense as mentioned by Dr. Ganapati is well known and accepted by all the health care professionals. However, we used the word “epidemiological characteristics” to describe various parameters/variables of reactions in relation to our large group of leprosy patients.

The risk factors and incidence over a period of time is discussed in relation to pauci- and multibacillary disease. It was beyond the scope of this hospital-based analysis to interpret results with respect to “communities” and “population groups.” Our results do provide important inputs for planning management of reactions both under institutional and field conditions, however being a hospital based study, both the incidence and severity of leprosy reactions may not be totally comparable to the situation in field. We have also given the data from the field studies vis-à-vis the hospital based figures for purposes of completeness and to help the readers/epidemiologists to draw their own conclusions.

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RÉSUMÉ

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Reprint requests: Hizir Ulvi, M.D., Department of Neurology, Medical Faculty, Atatürk University, Erzurum 25240, TURKEY. E-mail: hizirulvi@yahoo.com
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RESUMEN

El daño al sistema nervioso periférico es particularmente frecuente entre los pacientes con lepra. Los nervios trigémino y facial están entre los más frecuentemente afectados. El objetivo del estudio fue evaluar la eficacia del parpadeo reflejo como un método de diagnóstico de afección del nervio craneal en pacientes con lepra. Se estudiaron 37 pacientes (edad promedio: 38 años, 20 mujeres y 17 hombres) y 35 sujetos sanos apareados por edad (edad promedio 34 años, 20 mujeres y 15 hombres). Los reflejos de parpadeo se indujeron por estimulación eléctrica unilateral del nervio supraorbital y se evaluaron cuantitativamente 3 respuestas: el componente fásico ipsilateral temprano (R1), el componente tónico ipsilateral tardío (R2i), y el componente tónico contralateral (R2c). En todos los sujetos también se estudiaron los parámetros de conducción nerviosa. En comparación con los controles sanos, las latencias tanto del componente fásico ipsilateral temprano (R1) como de los componentes tónicos bilaterales tardíos (R2i y R2c) en los pacientes con lepra estuvieron significativamente prolongados. De 37 personas afectadas por la lepra, 22 (59%) mostraron anormalidades en la latencia R1, 28 (75%) en la latencia R2i, y 31 (83%) en la latencia R2c. No se observó correlación entre las latencias prolongadas y la duración de la enfermedad. Concluimos que la prueba de reflejos de parpadeo, la cual puede hacerse fácil- y rápidamente en un laboratorio de electromyografía usando equipo estándar, puede proporcionar información útil y objetiva para el diagnóstico de las lesiones en el nervio craneal y para determinar su grado de afectación.
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METHODS

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**TABLE 1. The demographic characteristics of patients and control subjects.**

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<td></td>
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<td></td>
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**THE FIGURE.** Examples of blink reflex in an adult control subject (upper) and a patient of similar age with leprosy (lower).
Correspondence

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The band-pass was set to 20–3000 Hz, sensitivity to 200 µV/cm, and the sweep velocity was 10 msec per division. Stimulation was performed with the cathode over the supraorbital foramen, with single stimuli on each side, consisting of square-wave pulses of 0.2 msec duration and 25 mA intensity. The ground electrode was positioned comfortably around the neck. Two recordings were obtained from both sides for each subject, with ten seconds or more between stimuli. Blink reflexes were obtained after unilateral electric stimulation of the supraorbital nerve for quantitative analysis of 3 responses, early ipsilateral phasic component (R1), late ipsilateral tonic component (R2i) and late contralateral tonic component (R2c). Latency was measured from the stimulus onset to the shortest initial deflection of the height R1 or R2 components. Amplitude was not important. Examples of blink reflex in a control subject and a patient are presented in Fig. 1.

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### RESULTS

The data from each group studied are summarized in Table 2. The latencies of both the ipsilateral early phasic component (R1) and bilateral late tonic components (R2i and R2c) in people affected by leprosy were significantly prolonged compared with the controls (Fig. 1). In general, abnormal R1 latency was less than that of R2. Out of 37 people affected by leprosy, 22 (59%) showed abnormalities R1 latency, 28 (75%) R2i latency and 31 (83%) R2c latency. No correlation was observed between prolonged latencies and the duration of disease.

**DISCUSSION**

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The blink reflex is a very practical, reproducible electrical response, which can be used in comparative clinical studies and experimental models (6, 18). It is an electrically induced glabellar response that has long been used in clinical neurology and is known to be a polisinaptic reflex with an afferent arc through sensory fibers of the trigeminal nerve and with an efferent arc through the motor fibers of the facial nerve (6, 7, 14). It has been most useful in the evaluation of lesions affecting the trigeminal

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Shetty, et al.\(^17\) reported that subclinical neuropathy may take place before clinical manifestations become apparent. Indeed, histopathological and immunocytochemical studies have demonstrated that nerve damage progresses from small unmyelinated to small myelinated and finally to large myelinated fibers\(^\text{15}\). The authors suggest that this electrophysiological study may well indicate subclinical neuropathy in these patients, since a significantly prolonged latency was not seen in the normal control subjects.

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In conclusion, we have observed markedly abnormal patterns of blink reflex in leprosy. The use of blink reflex testing which can be easily and rapidly performed in an EMG laboratory using standard equipment can provide useful, objective information for diagnosis and in determining the degree of cranial nerve lesions. However, future electrophysiological trials in active leprosy will provide further information for the use of this test in the early diagnosis of cranial nerve involvement in leprosy patients.

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I do agree with you that simplifying the classification system has been an invaluable tool to reach millions of untreated individuals and to lower prevalence, and it still remains a valid tool for public health. However it should not be confused with our endeavor to understand a disease through scientific research.

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Epidemiology of Leprosy

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Department of Dermatology, Venereology & Leprology
Postgraduate Institute of Medical Education and Research,
Chandigarh, India
Using the Blink Reflex as Measured by Electromyogram to Assess Cranial Nerve Involvement in People Affected by Leprosy

Hazir Ulvi, Remzi Yigiter, Tahir Yoldas, Recep Aygul, Asuman Orhan Varoglu, and Orhan Deniz

ABSTRACT

Damage to the peripheral nervous system is particularly frequent in leprosy patients. Trigeminal and facial nerves are among the most commonly affected. The aim of our study was to evaluate the efficacy of the blink reflex as a method for diagnosis of cranial nerve involvement in people affected by leprosy. We studied 37 affected people (mean age: 38 yrs, 20 female and 17 male) and 35 age-matched healthy subjects (mean age: 34 yrs, 20 female and 15 male). Blink reflexes were obtained after unilateral electrical stimulation of the supraorbital nerve for quantitative analysis of 3 responses, early ipsilateral phasic component (R1), late ipsilateral tonic component (R2i), and late contralateral tonic component (R2c). Nerve conduction parameters were studied in all subjects.

The latencies of both the ipsilateral early phasic component (R1) and bilateral late tonic components (R2i and R2c) in people affected by leprosy were significantly prolonged compared with the controls. Out of 37 people affected by leprosy, 22 (59%) showed abnormalities R1 latency, 28 (75%) R2i latency and 31 (83%) R2c latency. No correlation was observed between prolonged latencies and duration of the disease. We conclude that blink reflex testing, which can be easily and rapidly performed in an EMG laboratory using standard equipment, can provide useful and objective information for the diagnosis of cranial nerve lesions and for the determination of the degree thereof.

RÉSUMÉ

Les atteintes du système nerveux périphérique sont particulièrement fréquentes chez les patients lépreux. Les nerfs trijumeaux et faciaux sont parmi les nerfs les plus souvent affectés. Le but de cette étude était d’évaluer l’efficacité du réflexe de clignement comme méthode de diagnostic d’atteinte des nerfs crâniens chez les personnes affectées par la lèpre. Nous avons étudié 37 personnes affectées (âge moyen : 38 ans, 20 femmes et 17 hommes)
TO THE EDITOR:

Leprosy remains an important health problem worldwide. Leprosy was once widely distributed in Europe and Asia but now occurs mainly in resource-poor countries in tropical and warm temperate regions (1). According to the World Health Organization (WHO), at the beginning of 2003, the number of leprosy patients in the world was around 534,000, as reported by 110 countries. About 621,000 new cases were detected during 2002 (2). It is a very serious and mutilating disease in many parts of the world and diagnosis and therapy is the most important strategy for its control (3). All patients with leprosy have some degree of nerve involvement, making leprous neuritis undoubtedly the most common cause of treatable neuropathy in the world. Deformities from involvement of facial structures, eyes, nerves, bone, and skin can result in stigmatization and social ostracism. The diagnosis of leprosy is often missed by physicians in the United States, leading to delay of treatment during which progressive neuropathy, visual loss and deformity may occur (4). Diagnostic delay in leprosy can have serious neurological consequences for the patient. Lockwood, et al. (5) demonstrated that the median time from symptom onset to diagnosis was 1.8 yrs (0.2 to 15.2) and delayed in diagnosis occurred in 82% of cases in the United Kingdom. They also demonstrated that misdiagnosis as other dermatological and neurological conditions were important causes of delay 68% of patients in the UK were found to have nerve damage resulting in disability. Increased awareness among general practitioners and hospital specialists would lead to more rapid diagnosis, thus minimizing damage.
and disability. When detected and treated early, primary impairments may be reversible (21). For this reason, early recognition of leprosy is very important.

Trigeminal and facial nerves are among the most commonly affected in leprosy patients (11). Electrophysiological studies such as the blink reflex have been shown to be an effective method for revealing subclinical involvement of cranial nerves in generalised neuropathies (4, 5, 9, 11). The aim of our study was to evaluate the efficacy of the blink reflex as a method for diagnosis of cranial nerve involvement in people affected by leprosy.

METHODS

We studied 37 patients who had been treated for lepromatous leprosy (LL) with a mean age of 38 ± 17 yrs (range 23 yrs to 62 yrs; 20 female and 17 male), and 35 age-matched healthy volunteer subjects (control) with a mean age of 34 ± 12 yrs (range 24 yrs to 48 yrs; 20 female and 15 male). The demographic characteristics of patients and control subjects are presented in Table 1. Patients were hospitalized in Elazığ Leprosy Hospital for treatment and rehabilitation. The patients involved in this study had completed treatment and were cured of leprosy. The period between diagnosis of leprosy and study was 16.73 yrs. The patients and controls were carefully examined. All subjects in the study had a negative history and negative physical examination for central nervous system disease.

Exclusion Criteria:
• alcohol abuse
• cigarette consumption of over 10 cigarettes/day
• the presence of disease of the peripheral nervous system not related to leprosy
• tuberculoid and borderline leprosy (according to the Ridley-Jopling classification) (23)

Informed consent was obtained from all patients and controls before the investigations were carried out. Routine blood analysis was performed.

All subjects were studied using electromyogram (EMG) equipment (KEY-POINT, DANTEC, DENMARK). Following the standardized protocol proposed by Kimura, et al. (6) the supraorbital nerve in the supraorbital foramen was stimulated and the evoked responses from both orbicularis oculi muscles were recorded. Subjects were awake, in dorsal decubitus position, with room temperatures between 22 and 27°C, in a semi-darkened room. Surface platinum disc electrodes of 0.5 cm diameter were positioned as follows: channel 1, active electrode G1 was placed on the belly of the left orbicularis oculi muscle, 1 cm below the left lateral epicanthal point; reference electrode G2 was placed on the lateral surface of the nose; channel 2 was symmetrically positioned in relation to the channel 1 electrodes, on the right side. Fil-

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Robert H. Gelber, Ma. Victoria F. Balagon, and Roland V. Cellona

ABSTRACT

A group of multibacillary patients is clearly at high risk for relapse following 2-yr WHO-MDT. Relapse is largely confined to BL or LL patients with a high BI initially, and occurs long after the discontinuation of therapy. This important group of patients at risk for treatment failure presents several important issues: the need to identify those at risk and the operational requirements needed for their long term follow-up. Also, this group of patients might well benefit from an alternative antimicrobial regimen from the outset, as well as upon relapse.

RÉSUMÉ

En conclusion, il y a clairement un groupe de patients multibacillaires présentant un fort risque de rechute après le traitement polychimiothérapeutique (PCT) de 2 ans qui est recommandé par l’Organisation Mondiale de la Santé (OMS). Ces rechutes sont largement restreintes aux patients lépromateux borderline et polaires avec un index bactérien élevé et se produisent bien après l’arrêt du traitement. Ce groupe important à fort risque d’écueil thérapeutique soulève plusieurs questions sur le besoin d’identifier de façon prospective ces patients à risque, ainsi que sur les pré-requis opérationnels pour leur suivi à long terme. De plus, ce groupe de patients du début jusqu’à la rechute pourrait bien gagner à bénéficier d’un traitement antimicrobien différent du PCT de l’OMS.

RESUMEN

En conclusión, hay claramente un grupo de pacientes multibacilares que tienen un alto riesgo de recaída al completar la poli-quimioterapia (PQT) de 2 años recomendada por la
The World Health Organization (WHO) recently declared that relapse after Multi-Drug Therapy (MDT) was “low” (44). This has not been our (3) experience with 2-yr MDT in multibacillary (MB) leprosy, nor that of two other groups (17, 24), especially in those with an initially high bacteriologic index (BI). We recently reported that in MB patients followed up by our physician staff for 12 or more years after the completion of 2-yr MDT (3), 13 (9%) of 142 relapsed both clinically and bacteriologically, and in those MB patients with an initial BI of ≥2.7, 13 of 98 patients (13%) relapsed. Since that study was completed, 4 more relapses in that cohort have been confirmed, resulting in a present relapse rate of 16% (17 relapses in 106 patients). Also, in patients treated with 2 yr WHO-MDT, the Marchoux Study Group (Mali) (24) reported after a mean follow-up of just 5 yrs that both clinical and bacteriological relapse occurred in 20% (7/35) of MB patients, and 39% (7/18) with a pre-treatment BI ≥4+. Furthermore, in Agra, India (17), after 2 yr WHO-MDT and a mean follow-up of 4 yrs, bacteriologic relapse was detected in 7% (20/260) of MB patients and in 17% (18/107) of those with a pre-treatment BI greater ≥4+.

There is certainly contradictory data demonstrating that relapse rates following 2-yr WHO-MDT for MB leprosy is low (1, 5, 10, 27, 35, 36, 45). However, these studies are wanting on several grounds, particularly, that data was either based on questionnaires, a short duration of follow-up, or a low percentage of patients with a high bacterial burden. A very recent study from Kargiri, India (31) in smear positive MB patients followed up for 16.4 ± 1.8 yrs also showed a low relapse rate (2 of 84; 2%) in MB patients, but that study admittedly had features that would prejudice towards that outcome: only 12% of those patients had an initial BI ≥3+; approximately half of the MB patients put on therapy could not be followed up; these patients had a significantly greater percentage of borderline lepromatous (BL) and LL patients, and a higher initial BI; many of these patients had received prior dapsone monotherapy, and more than half of the patients received more than 2 yrs of MDT, being treated until smear negativity. Nonetheless, in that study, 20% of patients with a BI ≥3+ relapsed.

**Cebu relapse experience.** In Cebu, our relapse definition required both new skin lesions and increased BI of 2+ in one or more smear sites than had been found on the last such evaluation. Our MB relapse experience after 2-yr WHO-MDT of 22 cases (some followed in the field) comprises the largest such group detected to date and the longest duration of follow-up in patients with an initially high BI. The experience reveals several clinical features.

**Characteristics of lesions on relapses.** Relapsed patients commonly present new lesions of the maculo-papular type and some localized infiltration on the body and extremities. Most localized infiltration, however, arose at old affected areas that had already subsided. A few plaques and nodules presented as new lesions. The lesions presented by the 22 relapsed patients are summarized in Table 1. In all but 1 of our relapses, the BI had been 0 at 6 smear sites at the examination prior to relapse detection, indicating that these are indeed relapsed cases rather than treatment failures. The BI at the highest relapse site was generally high: 5+ (17), 4+ (3), 3+ (1) and 2+ (1). Also, very frequently, the earlobes were involved and infiltrated, with positive smears. Of the 22 relapsed patients, 12 (54.5%) had involvement of both earlobes with positive smears. An additional 5 patients (22.7%) had positive smears of 1 earlobe only. Involvement of 1 or both earlobes together were found in 17
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of the 22 relapsed patients (77.3%). Thus, in addition to new skin lesions, more than three quarters of our relapses developed bacteriologic relapse in one or both earlobes.

*Leprosy type and initial BI.* All of the 22 relapsed patients were found to be clinically and histopathologically BL (10) or LL (12), in equal proportion to that found at the start of treatment. This implies that the risk of relapse is the same in BL and LL. No relapses were found in any of the borderline tuberculoid (BT) or borderline borderline (BB) patients followed up by physicians in this cohort.

*Years elapsed after MDT when relapses occurred.* The earliest of all relapses was detected 6 yrs after the completion of MDT. We found that the risk of relapse is nearly twice as much 10 yrs after treatment than prior to that time. This experience is summarized in Table 2.

*Sex and age.* The male to female ratio of the study population of MB patients was roughly 3:1. Of the 22 relapsed cases, 19 were males and 3 females, or a ratio of roughly 6:1. Though not statistically significant (p = 0.13), this suggests that males may have as much as two times the risk of relapse compared to females.

The age of the 22 relapsed patients ranged from 9 to 55 yrs at the start of treatment. We found a more or less equal proportion of relapses in patients initially treated in the young age group, 9 to 20 yrs old (8), middle age, 21 to 33 yrs old (7). This suggests that the risk of relapse is the same for leprosy patients independent of age.

*BI after 2-Year WHO-MDT.* Of the 22 relapses, 9 patients (roughly 41%) had already achieved negative smears after the 2-yr WHO-MDT treatment. This finding indicates that a negative smear after the end of treatment does not guarantee against future relapse.

However, all but one of the patients in the study eventually had negative smears in the 5 yrs after the end of treatment. In other words, almost all the relapsed patients passed through a period of negative smears before relapsing.

**Effects of steroids on relapses.** Of the 268 MB patients who were treated with the 2-yr WHO-MDT by the Cebu Skin Clinic (many with a follow-up of less than 12 yrs), 149 were given steroids because of lepra reactions, while 119 were not.

- Of the 149 given steroids, 9 relapsed (roughly 6.0%)
- Of the 119 with no steroids, 8 relapsed (roughly 6.7%)

The risk of relapse therefore appears to be the same for those given steroids during treatment and those that were not.

*M. leprae sensitivity.* The organisms taken from the biopsies of 16 clinically relapsed patients grew in the groups of mice not given any anti-leprosy drugs, confirming their viability and proof of relapse, while the results of 6 others are still pending. All of the organisms from the 16 patients did not grow in the groups of mice

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**Table 1. The lesions presented by the 22 relapsed patients.**

<table>
<thead>
<tr>
<th>Type of Lesion</th>
<th>Number of Relapses</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Macules, papules, localized infiltration</td>
<td>Found in 6 relapses</td>
</tr>
<tr>
<td>b. Papules, localized infiltration</td>
<td>Found in 2 relapses</td>
</tr>
<tr>
<td>c. Plaques only</td>
<td>Found in 1 relapse</td>
</tr>
<tr>
<td>d. Macules, nodules</td>
<td>Found in 1 relapse</td>
</tr>
<tr>
<td>e. Macules, papules</td>
<td>Found in 2 relapses</td>
</tr>
<tr>
<td>f. Nodules, localized infiltration</td>
<td>Found in 2 relapses</td>
</tr>
<tr>
<td>g. Localized infiltration</td>
<td>Found in 3 relapses</td>
</tr>
<tr>
<td>h. Macules, localized infiltration</td>
<td>Found in 3 relapses</td>
</tr>
<tr>
<td>i. Erythematous infiltration</td>
<td>Found in 2 relapses</td>
</tr>
</tbody>
</table>

**Table 2. Years elapsed after MDT when relapses occurred.**

<table>
<thead>
<tr>
<th>Time After MDT</th>
<th>Number of Relapses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 5 yrs</td>
<td>None relapsed</td>
</tr>
<tr>
<td>6 to 9 yrs</td>
<td>8 patients relapsed</td>
</tr>
<tr>
<td>10 yrs and above</td>
<td>14 patients relapsed</td>
</tr>
</tbody>
</table>
given rifampicin in their diets and clofazimine by gastric gavage. Two patients had organisms that grew in the groups of mice given the lowest concentration of dapsone (0.0001%) but not higher concentrations (“partially resistant”), and 1 patient harbored *M. leprae* that grew in mice fed 0.01% dapsone diet (“fully resistant.”) These 2 patients are therefore mildly resistant to dapsone. It is noteworthy that Matsumuka has found no genetic mutation towards dapsone resistance in any of our 16 relapsed patients (personal communication). This discrepancy between that analysis and the results in the mouse footpad requires our further study. In any event, MDT does not appear to result in drug resistant relapse, nor, in particular, multidrug-resistant relapse.

**IMPLICATIONS**

Our relapse experience is particularly noteworthy in that all of our relapses occurred in BL or LL patients, and all but 1 relapse was found in patients with a pre-treatment BI ≥2.7 (1). In Mali (24) and in Agra, India (17), 25 of the 27 relapses (93%) occurred in patients with a pre-treatment BI ≥4+. In Karigiri, India (31) the two relapsed patients were among the 12% of those in that trial with an initial B.I. ≥3+, having a B.I. of 3.0 and 3.5. Thus, in all four studies a high initial BI was found to be a significant risk factor for subsequent relapse. Until the last decade, skin smears and the importance of precise clinical and pathologic classification across the leprosy spectrum were viewed as critical tools for choosing the appropriate regimen of treatment, as well as to predict response to therapy and subsequent reactional states. Today skin biopsy is practiced in a small minority of endemic locales, and an even smaller proportion of those where it is done are experienced with the interpretative skills required for placing a patient in the proper pathologic designation. Also, skill at skin smears has waned to the point that it has been found inaccurate in most locales, such that the WHO (46) no longer advocates its use in classifying patients as MB or PB. This distinction is now determined simply by counting the number of skin lesions, MB being 5 or more and PB being less. Such an approach is especially wanting and hard to support insofar as BL leprosy can often present with less than 5 skin lesions and at times but 1. Of utmost relevance to the lost use of skin biopsies and skin smears is that without them identification of those MB patients at high risk for relapse is largely unavailable worldwide.

Another observation from our relapse experience in the Philippines is that MB relapse occurs long after the discontinuation of 2-yr WHO-MDT. Of our 22 relapses, the first was detected 6 yrs after the discontinuation of therapy, and 14 were found 10 or more years after therapy ended. A similar experience was reported by Pattyn (23) after a 6 week intensive quadruple regimen (rifampin, ofloxacin, dapsone, and minocycline), wherein relapses were first detected at 6 yrs with a doubling in years 8 and 9. The Marchoux Study Group (24) found that MB relapse after 2-yr WHO-MDT occurred at a mean of 6 ± 1.5 yrs after the completion of therapy, while in Agra, India (17), where relapses were defined on bacteriologic grounds about 30% of the time without concommitant clinical manifestations, the average time to relapse was 4 yrs. Also in Agra, relapses were significantly higher in those MB patients followed up greater than 4 yrs than in those followed up for a lesser duration (17). Finally, in Karigiri, India, the two relapses detected were found 14 and 15 yrs after the completion of therapy (31). These experiences of late relapse following treatment for MB leprosy are in sharp contrast to the experience with short-course therapy for pulmonary tuberculosis, wherein 90 of relapses occur within the first year post-therapy. This has operationally led to the general policy of follow-up for patients with active pulmonary tuberculosis in the United States to be completed 1 yr after discontinuation of therapy. In any event, after the completion of WHO-MDT, there is currently no recommendation for follow-up at all and certainly not for the decade or longer needed to detect clinical and bacteriologic relapse (46). Perhaps the dramatically longer relapse interval in MB leprosy, as compared to pulmonary tuberculosis, is a reflection of the relative doubling time of the different mycobacteria, i.e., one day for *M. tuberculosis* and 14 days for *M. leprae*. Another possible factor is the higher bacterial load that may need to be attained in leprosy.
prior to the development of signs and symptoms.

In many control programs today, once MDT is concluded for leprosy a patient is no longer considered a case and follow-up is not mandated. Thus, relapses in leprosy are largely left to self-referral and not to active and organized interventional follow-up by the health services. This, coupled with the long interval between the completion of MDT and the appearance of relapse, will likely result in a delay of relapse diagnosis and an increased rate of relapse morbidity. This potential is further heightened by our observations that relapse frequency in the Philippines was found to be lower (3%) when patient follow-up was conducted by even well-trained and experienced leprosy health workers rather than our physician staff (4). Worldwide relapse detection is further compounded when that diagnosis, is left to the general health services, which is now largely becoming the case. Fortunately, in our relapse cases, relapse was not associated with neurologic deterioration. Perhaps this salutary result was in part a function of prolonged, active, annual follow-up and by trained leprologists. Neither of these conditions are reasonable expectations in most endemic locales today, thereby again increasing the potential risk of seriously delaying the diagnosis and the further possibility of increasing the risk of associated increasing peripheral neuropathy.

The relapse rate we and two other groups have noted is clearly high and would be considered unacceptable so in the treatment of pulmonary tuberculosis, where regimens which result in relapse rates greater than 5% have been rejected as unacceptable (2). Though there are no significant data on relapse following the current WHO recommended 1 yr (46) or 6 month MDT trials (43), there is little doubt that therapy for such short durations would result in relapse rates at least as high as 2-yr MDT. These regimens are therefore clearly difficult to support. Importantly, because the planned follow-up duration of the current WHO 6 month MDT (43) is only 5 yrs, a period shorter than our earliest relapse following 2-yr MDT, the follow-up in that study requires expansion to at least 15 yrs if meaningful data on relapse rates are to be obtained. Furthermore, we (unpublished) have found that both Type 1 and Type 2 lepra reactions, occur in a significantly larger percentage of patients, with greater frequency and for longer duration following 1-yr MDT as opposed to 2-yr MDT. This important additional morbidity and particularly its propensity for increased nerve function impairment provides additional evidence to reject 1-yr MDT for MB patients.

The issue of whether relapses in leprosy are due to reactivation of persisting organisms or to reinfection is unresolved to date. In fact, lepromatous patients regularly harbor viable persistent M. leprae following prolonged chemotherapy, in over half of patients treated with 10 or more years of dapson (41) or 5 yrs of rifampin (42). In tuberculosis the technology to distinguish strain variability has been available now for several years; while the bulk of tuberculosis relapse in the immunocompetent host has been established to be due to reactivation, there is a considerable percentage of relapses in AIDS patients that are, in fact, reinfection (40). In this regard, it is noteworthy that while BL patients may gain specific M. leprae immune-reactivity after effective chemotherapy, LL patients remain anergic (6). It is only now in leprosy that genetic polymorphisms amongst M. leprae strains have been detected (18, 19, 28, 39), and we are currently examining this issue. If leprosy relapse is, in fact, reactivation, reinfection, or at times both, data on this issue should be soon available.

**ALTERNATIVES TO 2-YEAR WHO-MDT FOR MB LEPROSY**

Considerations for improving 2-yr WHO-MDT include: (i) extending the duration of MDT and/or adding an initial intensive phase of bactericidal therapy, (ii) the use of combinations of newer and more bactericidal drugs, and (iii) life-long therapy.

(i) In several locales, the duration of MDT has been extended and/or combined with the incorporation of an earlier, more intensive regimen and with improved relapse rates. In Agra (17), when patients with a pre-treatment BI of 4+ or greater were treated with 2-yr WHO-MDT a relapse rate of 17% was obtained, while when MDT was extended to smear negativity (on average 5 yrs) the relapse rate was reduced to 4%. In Malaysia, since 1986 the standard
regimen for MB leprosy has been initial hospitalization with daily observed therapy (including dapsone, clofazimine, rifampin) for 1 month, followed by WHO-MDT for 5 yrs. On this regimen, no relapses have been found in several hundred patients (personal communication, Majid, Azmon). In Bhutan, where many patients had been on dapsone monotherapy (some for prolonged times), patients were hospitalized and given daily dapsone and rifampin for 1 month, followed by dapsone alone for 1 yr. Unfortunately, follow-up of this experience is currently unavailable. In the early 1990s, the WHO embarked on a blinded comparative multi-centered trial of 4 regimens: (i) Daily rifampin and ofloxacin for one month, (ii) Regimen 1 followed by standard 1-yr WHO-MDT, (iii) 1-yr WHO-MDT, and (iv) 2-yr WHO-MDT. In Cebu, several early relapses were observed at one of the trial sites, particularly with Regimen 1. At least in Cebu, the 189 patients from that study have been on active annual follow-up for a long enough period that meaningful comparative relapse rates might become available. However, the WHO has not chosen to break the treatment code for late relapses in this study, making it impossible to determine the relative efficacy of the four treatment regimens, and it is not supporting the follow-up.

(ii) To date, only rifampin, dapsone, and clofazimine, the 3 components of WHO-MDT, have been utilized to any considerable extent to treat leprosy. Of these 3 antimicrobials, only rifampin has proved bactericidal in leprosy patients (34, 38). On the other hand, agents from 3 other classes of antimicrobials, tetracyclines (minocycline) (7, 11, 14, 25, 26), macrolides (clarithromycin) (4, 9, 15, 25, 26), and fluoroquinolones (ofloxacin and ofloxacin) (8, 20, 21, 22, 30) are bactericidal for M. leprae both in mice and leprosy patients. Though the WHO (46) has advocated single doses of rifampin, ofloxacin, and minocycline (ROM) for PB leprosy, and this has appeared effective in those with single lesions (32), combinations of these newer agents with rifampin in leprosy patients have not been tested to any considerable extent, and none with long term follow-up. Though Pattyn reported his previously described 4-drug regimen, 20% of patients relapsed (32). However, in that trial the duration of therapy was only 6 weeks and the regimen might have proved superior had it been maintained for a longer duration.

In the mouse model, there have been few studies comparing combinations of antimicrobials (37). One study (16) found that 2 and 3 drug combinations were generally additive or synergistic. Studies of regimens in the immunosuppressed, neonatally-thymectomized Lewis rat (NTLR) (12) appeared to be particularly useful in predicting which combinations might be especially effective in preventing relapse in MB patients. Heavily infected NTLR were treated with various antimicrobial combinations and evaluated for the presence of any persisting viable M. leprae one year after the completion of therapy by subsequent inoculation of both mice and NTLR. Several anti-microbial combinations appeared particularly effective, but only rifampin plus minocycline uniformly killed all M. leprae. Perhaps this combination deserves special attention.

(iii) Lifelong therapy for MB leprosy, principally dapsone monotherapy, was the norm and was highly successful for most of the latter half of the 20th Century. On dapsone monotherapy skin lesions resolved, neurologic deterioration ceased, patients became bacteriological negative and relapse, principally from dapsone-resistance organisms, was rare (2.5%) (13, 29). Dapsone monotherapy, moreover, is both inexpensive and has few side-effects, especially after the first few months of treatment. In certain locales drug supplies and long-term compliance are significant issues, but many patients, especially in the developed world, are on life-long medication for such things as diabetes mellitus hypertension, stroke prevention, hypercholesterolemia, gout, etc., wherein single daily doses of medication are found generally acceptable and ordinarily maintained. Why not consider lifelong therapy for a subset of MB patients?

REFERENCES


The Report of the 6th World Health Organization Technical Advisory Group on Elimination of Leprosy contains much interesting material, and we strongly recommend that all ILA members and other interested persons read it carefully (WHO/ CDS/ CPE/ CEE/ 2004.41) (http://www.who.int/lep/). Section 11, entitled “Validation of diagnosis of newly detected cases,” particularly caught our attention.

This section reports the results of a study of the accuracy of the diagnosis of leprosy in clinics in several regions of India. The study was conducted by having two individuals with substantial clinical experience in leprosy review the patients initially evaluated by health care workers at the clinics, to ascertain the accuracy of the original diagnosis. The study reports, among other things, that over 30% of the patients in the Delhi clinics were “non-existent patients” because they could not be confirmed to live at the addresses given, which in some cases were fictitious addresses. Based on this and related information, the study concluded that leprosy was greatly over-diagnosed in these clinics, and a policy recommendation is made that such patients should be removed from the leprosy register.

Two possible reasons for this finding are offered: “[1] these were either fictitious cases or [2] individuals who gave a misleading address.” This first explanation is favored by the authors of the report, who have then labelled these individuals as “non-existent patients.”

If this explanation is correct, i.e., that these are fictitious cases, it implies that nearly 1/3 of all patients in the Delhi clinics have spent most of a day uselessly queuing up in lines in hot, crowded clinics in order to obtain the diagnosis of leprosy, a diagnosis which brings them no gain but, rather,
may consign them to lifelong stigma, ostracism, family rejection, and reduced employment and earning ability. Most of these people depend on each day’s earnings to provide food for themselves and their families for that day. While it is possible that one or two deranged individuals may waste their days in clinics pretending to have leprosy, it is clearly illogical to conclude that nearly 1/3 of the patients in the Delhi clinics do so. Yet this report implies that this is the most likely explanation and then gives them the pejorative label of “non-existent patients.” Finally, based on this conclusion, one of the main recommendations in the next section (11.2), is that: “Non-existent patients should be removed from the leprosy register.”

In contrast, the second explanation (i.e., individuals gave misleading addresses) is a phenomenon well-known to all leprosy control programs: individuals move to another city, another region, or even another country when they suspect the diagnosis, to avoid the possibility that friends or family will learn that they have leprosy. It is ludicrous to suggest that 1/3 of patients are “faking” leprosy, while it is obvious that these individuals are concealing the diagnosis from their own communities. These, then, are not “non-existent patients” but non-existent addresses for real patients needing medical care. This also indicates that there is much more work to be done, not less, in finding and treating those patients who gave fictitious addresses. If 1/3 of the patients are evading follow-up, it is highly likely that a substantial number of them are capable of transmitting the disease to their close contacts. Rather than expend energy on the avoidance of over-treatment, more energy must be expended in delivering appropriate treatment.

What can be done to avoid such over-interpretation of information and formulation of premature recommendations? One suggestion is that more caution be exercised in accepting evidence put forward, and in drawing conclusions from that evidence. This is one area in which medical and scientific journals can play a valuable role.

Everyone who has submitted a research report to the Journal, or to any other peer-reviewed journal, is familiar with the rigorous process of review, requests to revise and re-submit a paper to make it clearer, to improve its statistical treatment of the data, and to avoid over-interpretation of results and over-reaching for conclusions. In spite of its flaws, this process is regarded by the worldwide medical and scientific community as a necessary step in the larger process of ensuring that new treatments or treatment policies are based on sound evidence and that their implementation is ethical. Clearly, recommendations for treatment of leprosy should be given no less care and consideration.

An example of such an approach is the comprehensive set of recommendations based on careful examination of the evidence concerning diagnosis, chemotherapy, and epidemiology of leprosy, contained in the Report of the ILA Technical Forum (2). The evidence presented in that report was discussed in many of the symposia and workshops of the 16th International Leprosy Congress, and after a spirited debate over several aspects of the recommendations, the ILA General Assembly passed a Resolution calling on “all stakeholders (including national governments, international organizations and non-governmental organizations) to review their recommendations in the light of this report” (emphasis mine) (1). This careful, measured approach still seems the wiser path.

The accurate diagnosis and classification of leprosy has always been essential in the delivery of the best care to individual patients and in formulating the best health policy in individual countries. In a previous issue we addressed concerns over the inadequacies of some classification schemes for research studies (3). We now agree with the goal of the validation study described in the 6th WHO TAG report, and hope that a more careful study can be performed to address the important issues raised.

In the meantime, we hope that Ministries of Health around the world, and their leprosy control officers, will not be too eager to accept the Report’s premature recommendations related to those patients who are not easily located. It would be most unfortunate to adopt policies to disregard these patients, both because the patients and their contacts are not well served by such a policy, and because the resulting numbers in the registry may then seriously under-
estimate the true extent of the disease. Certainly we should not condone the addition of any more pejorative labels such as “non-existent patients” to describe the worried, wary individuals whose trust the leprosy control workers must gain in order to give them the best medical care.

—DMS

REFERENCES
OBITUARY

PROF. LUIZ MARINO BECHELLI
1912–2004

Prof. Luiz Marino Bechelli attended medical school at University of São Paulo where he graduated in 1933. In 1957, he was invited to organize the Department of Dermatology of the new School of Medicine at Ribeirão Preto – University of São Paulo. He then decided to abandon his private practice and devote his life to dermatology, both as a teacher and a researcher. In order to improve his knowledge, he became a post-graduate fellow at the New York Skin and Cancer Center at Columbia University, the Western Reserve University in Cleveland and visited many institutions such as the Hôpital St. Louis (Paris-France). While a Professor at the University of São Paulo, one of his outstanding contributions was the publication of a comprehensive *Textbook of Dermatology* in 1960, which had its 6th edition in 1988.

Prof. Bechelli joined leprosy work at the Department of Leprosy of São Paulo as a trainee and later became Chief of Epidemiology. Intense activity as a researcher and as a practitioner lead to the publication of more than 300 papers in this field. Together with Dr. Abrahão Rotberg, he published the well known *Brazilian Treatise on Leprosy* and the *Compendium of Leprology*. He also served as Chief of the Leprosy Unit of the World Health Organization in Geneva from December 1961 to June, 1972.

His services to the cause of leprosy and dermatology has granted him membership in many international societies such as *Association des Léprologues de langue Française, Sociedade de Dermatologia y Sifiligrafia, Sociedade Cubana de Dermatologia y Sifiligrafia, Sociedade Brasileira de Hansenologia, Société de Pathologie Exotique, Sociedade Argentina de Dermatologia, Colégio Ibero-Latino-Americano de Dermatologia, and Sociedade Brasileira de Dermatologia*. He also acted as a contributing editor to the the *INTERNATIONAL JOURNAL OF LEPROSY*.

After his compulsory retirement from the School of Medicine at U.S.P., he worked for more 14 yrs without payment, explaining: “I worked free of charge in order to compensate the University for the period I dedicated to WHO.”

Prof. Bechelli was a noble example of life and dedication to patients, friends, and colleagues, and he will always be remembered.

—Norma T. Foss, M.D.

*President*,
*Brazilian Society of Hansenology*

—Marcos Virmond, M.D.

*ILA Vice-President for the Americas*

The Wellesley Bailey awards are unique. In June 2005, two remarkable people who have had leprosy and faced significant challenges from the disease, will be presented with the fourth Wellesley Bailey Award. There is no other international award which acknowledges the accomplishments of those who have been affected by leprosy.

Created in 1999 to celebrate the life and work of Mr. Wellesley Bailey, founder of The Leprosy Mission, the Wellesley Bailey awards honour those who have made extraordinary contributions to society through overcoming the social stigma and physical challenges of leprosy.

A former recipient of the awards said “Leprosy is a ruthless thief, which first, turns off all the lights before you notice that there is an intruder. Then in the total blackout it viciously created, it robs you of every single irreplaceable treasure you possess.”

The recipients will have been active members in their community for at least five yrs and will be champions for the cause of leprosy through their achievements.

Each Award includes a prize of £1000 and a presentation plaque. The recipients will be invited to travel to Dublin, with a supporter of their choice, to receive the Award at an awards ceremony in the first week of June 2005.

Nominations for the 4th Wellesley Bailey awards. These unique international awards acknowledge the accomplishments of those who have made extraordinary contributions to society despite the challenges of leprosy.

Recipients must have personally experienced leprosy, will have been active members of their community for at least five years and champions for the cause of leprosy.

Each award includes a prize of £1000 and a presentation plaque. The recipient will be invited to travel to Dublin for a special awards ceremony in the first week of June 2005.

Nominations must be submitted on the official form available from Glynis Forbes. The deadline for nominations is 10 January 2005.


The following excerpt is from a press release from the Nippon Foundation, dated Aug. 16, 2004, forwarded to the JOURNAL by Dr. P. K. Gopal, President, IDEA International.

August 9, Geneva—Today, at the European Headquarters of the United Nations, the 56th Sub-Commission on Human Rights reached a consensus to take up the question of human rights violations visited upon leprosy patients, cured people and their families.

The sub-committee adopted a formal resolution to study the issue. Under the auspices of this resolution, members of the commission will conduct an investigation and produce a working paper on leprosy and human rights. This paper will then be presented at the 57th meeting of the sub-committee.
There are roughly 500,000 leprosy patients and 20 million former patients around the world. When family members are taken into account, over 100 million people are suffering from the needless discrimination related to leprosy. Leprosy is incorrectly thought by many to be hereditary or a supernatural punishment, and is thus greatly feared. However, with multi-drug therapy, this “incurable disease” has become completely curable. In spite of this fact, individuals affected by the disease must still suffer discrimination that limits their ability to study, marry or find work. Even after death, families often face lasting social discrimination.

The full text of the resolution follows:

COMMISSION ON HUMAN RIGHTS
Sub-Commission on the Promotion and Protection of Human Rights

Fifty-sixth session
Agenda item 5

PREVENTION OF DISCRIMINATION
Draft resolution 2004/
Discrimination against leprosy victims and their families

The Sub-Commission on the Promotion and Protection of Human Rights,

Recalling article 1 of the Universal Declaration of Human Rights, which stipulates that all human beings are born free and equal in dignity and rights, Recalling also article 2 of the Universal Declaration, which provides that everyone is entitled to all the rights and freedoms set forth therein without distinction of any kind, such as race, color, sex, language, religion, political or other opinion, national or social origin, property, birth or other status, Recalling further article 5 of the Universal Declaration, which provides that no one shall be subjected to torture or to cruel, inhuman or degrading treatment or punishment, Concerned that millions of people suffer from discrimination resulting from physical and mental illness or handicap, Concerned in particular that tens of millions of people suffer not only from leprosy as a disease, which is scientifically and medically proven to be curable and manageable, but also from political, legal, economic or social discrimination and isolation as a result of misunderstanding and indifference, and lack of legislative or administrative measures to prohibit such discrimination and to protect and remedy the victims, Requests Mr. Yozo Yokota to prepare, without financial implications, a preliminary working paper on discrimination against leprosy victims and their families, to be submitted to the fifty-seventh session of the Sub-Commission under the agenda item entitled “Prevention of discrimination and protection of minorities.”

Notice. The First Libyan Dermatology and Venereology Society Congress

The Libyan Dermatology and Venereology Society is honored with the participation of the international experts (from U.S.A., U.K., France, Malta, Tunis, Egypt, Syria, and Yemen), Libyan dermatologists and doctors from related specialties who gathered at the first Libyan Dermatology and Venereology Society Congress held at Tibesti hotel, Benghazi from the 23rd to 25th of July 2004. The theme of the Congress was “For healthy skin free from diseases.” Congress president and Organizing Committee Chairman was Dr. Gamal A. Duweb, M.D. Scientific Committee Chairman was Dr. Mohamed Benghazil, M.D.

The scientific program included a variety of learning styles such as plenary lectures, free communications, case presentations, a and poster session, covering most of the common dermatological diseases including leprosy, leishmaniasis, mycotic infections, psoriasis, acne, vitiligo, eczema and genodermatosis.

During the leprosy session, Professor W. Cains Smith talked about recent advances in prevalence and treatment of leprosy and its complications; he pointed out that since 1985, the global prevalence of leprosy has fallen by almost 90% and more than 13 million have been cured, which has been achieved by the implementation of intensive courses of multi-drug therapy (MDT). Also, the number of new cases detected has remained static at around 700,000/yr.

Professor Paul Fine, in his lecture on the elimination of leprosy: Progress and problems, clarified that programs emphasize the prevalence data and changes in case finding methods in treatment regimens, making assessment of trends difficult. Fine also
mentioned that the natural history of *Mycobacterium leprae* infection and the leprosy disease are still poorly understood, but it is evident that infection and disease will remain a problem in local populations in several parts of the world for the feasible future.

Professor Yassin Alqubati discussed the social effects of leprosy stigma among Arab countries, and the importance of community health education in treating leprosy patients. During the free communications and poster session, local data analysis of 92 patients registered at a leprosy clinic in Benghazi was presented and showed that lepromatous leprosy (LL) was the most common clinical type; LL patients were 64% of the total, and 89.1% of those had completed MDT treatment courses.

Discussion with the experts concluded that the number of new cases is low. The difficulties of leprosy and contactant tracing was discussed in detail, as was the regular screening of the new cases and their families. Possibly recommending regular screening for the contactant of registered cases was also discussed.

in the Context of HIV/AIDS” will be held on Robben Island, South Africa, from Feb. 4–6, 2005. For information on the conference and how to register, please contact Jo Robertson, jo.robertson@history-of-medicine.oxford.ac.uk or Anwei Law, alaw@idealeprosydignity.org

Notice. from Brazil,

Brazilian Seminar on Hansen’s Disease. The Brazilian Society of Hansenology held its second national seminar on July 23–24, 2004 in Ribeirão Preto. The meeting was attended by participants of a ample array of representative of health workers from Brazil, including physical therapists, psychologists and social workers. The president of the Brazilian Society of Hansenology, Prof. Norma T. Foss, was in charge of the organizing committee and conducted the opening and closing sessions. Invited guest speaker was Dr. P. K. Das from the Amsterdam Medical Center, The Netherlands.

10th Brazilian Congress of Hansenology. The Brazilian Society of Hansenology will to hold its 10th Congress next November, 2005 in João Pessoa, Paraiba State. The local organizing committee is headed by Dr. Francisca Estrela. The last national Congress was held together with the 16th ILA Congress in Salvador, August 2002. Many topics are planned for discussion in this meeting, including the achievements of the efforts towards elimination of leprosy in Brazil.

Brazilian National Leprosy Control Program under new leadership.

Dr. Rosa Castalia França Ribeiro Soares has been appointed as the new head of the leprosy control program in Brazil. Dr. Soares is a specialist in public health and has worked for many years in the immunization program in Brazil. She has also had considerable experience in leprosy while chief of the control program in the State of Bahia. Dr. Soares is now leading a new program in the Ministry of Health—the National Program of Elimination of Leprosy—the main goal of which is to achieve elimination of leprosy by improving and expanding diagnosis, treatment and follow-up of cases in the basic health system network. Integration and partnership with other organizations has been identified as essential to achieve the goal of elimination and in this regard many partners have joined the Program such as ILEP, Children Pastoral, MORHAN, and CONSASEMES, among others.

Notice. New AIFO Website.
http://www.aifo.it/english/index.htm
Among the new additions to this web-page are the following:
1. A page on PHM in Italy and information about PHA-II in Cuenca next year. This page includes links to women’s access to health and million signature campaigns.
2. Online books, documents and reports related to leprosy, disability, primary health care, etc.
3. Some online learning courses on leprosy and tropical diseases.
4. Online exhibitions—at present there is just one exhibition (on Angola) but hopefully new ones will be added soon.

—Dr. Sunil Deepak

AIFO, Italy

Notice. from India,

Schieffelin Leprosy Research and Training Centre Golden Jubilee.

As a part of its 50th anniversary celebrations, SLR&TC, Karigiri, India, hosted a Scientific Workshop entitled, “Leprosy Research: Challenges of the Decade,” from August 16–18, 2004, at Karigiri. The Workshop was hosted by Dr. V. P. Macaden, Vice Chairman of the Centre’s Board of Governors. Dr. Margaret Brand was the featured guest and keynote speaker, and other dignitaries in attendance included Dr. N. K. Ganguy, Directory General, ICMR, and Dr. S. Habayeb, WHO Representative to India. The workshops included state of the art presentations on both basic and clinical aspects of leprosy, and discussion groups composed of physicians and scientists attending from India, Nepal, the Netherlands, the U.K., Kenya, Brazil, and the United States. The participants worked in small groups to develop research proposals, and were encouraged to continue to collaborate in the development of these proposals so that the research projects may be initiated in the near future.

Even in an age of masterful clinicians, Sir Jonathan Hutchinson was exceptional. In addition to his clinical work, Sir Jonathan was a prolific writer serving as editor for a time of the British Medical Journal . . . and, most incredibly, from 1889–1900 single-handedly writing every article in his 1-man journal, the Archives of Clinical Surgery. Sir Jonathan was a medical polymath, an expert in ophthalmology, neurology, pathology, surgery, and dermatology. It is in syphilology where his eponymous fame is most enduring . . . having seen over 1 million cases (1) of syphilis . . .

Unfortunately appended to his illustrious career will always be the enduring embarrassment of his idée fixe—that leprosy is caused by eating rotten fish. Notwithstanding the questionable geography or the less-than-obvious logical dietary leap, for the rest of his life Hutchinson espoused his doctrine despite universal rejection by contemporary leprologists. Even the discovery of the leprosy bacillus by his good friend Gerhard Hansen in 1874 couldn’t dissuade Hutchinson. Hutchinson was mildly non-plused when no one could discover [the bacillus] in any fish . . . Hutchinson confessed that one reason he never sought to experimentally test his theory was “the assured conviction that the general facts were overwhelmingly conclusive and that they needed only to be clearly set forth.” Indeed the only reason Hutchinson’s theory gained any circulation at all, if never actual acceptance, was because of his otherwise impeccable reputation.—[Condensed from the essay. DMS] Archives of Dermatology


Although the prevalence rate of leprosy in the Republic of Yemen has dropped below the WHO elimination level of less than one
case per 10,000 of the population, it is still regarded as a serious public health problem calling for continued vigilance, notably in the detection and treatment of hidden and undiagnosed cases. In the past, religious misinterpretation has generated adverse behaviour patterns towards people affected by leprosy, characterized by aggression, negligence and isolation. Until about 1982, following a visit of a leprologist (Dr. S. K. Noordeen) from the World Health Organization, there was no leprosy control program and attempts to establish one remained ineffective until in 1989, when an agreement was signed between the Ministry of Public Health and Population and the German Leprosy Relief Association. This led to the development of a leprosy control program in four governorates, later extended to the rest of the country. This paper describes the progress made in the control of leprosy in the Yemen, 1989–2003, by the Ministry of Health and Population and the GLRA, in association with two local societies.—Authors’ Abstract


During the past 10 years palaeomicrobiology, a new scientific discipline, has developed. The study of ancient pathogens by direct detection of their DNA has answered several historical questions and shown changes to pathogens over time. However, ancient DNA (aDNA) continues to be controversial and great care is needed to provide valid data. Here we review the most successful application of the technology, which is the study of tuberculosis. This has provided direct support for the current theory of Mycobacterium tuberculosis evolution, and suggests areas of investigation for the interaction of M. tuberculosis with its host.—Authors’ Abstract


16S rRNA gene sequence analysis provided evidence for two different mycobacterial species, Mycobacterium leprae-murium and a potentially novel species, as causative agents of “feline leprosy.” Comparison of 16S rRNA gene sequence data obtained for M. leprae-murium and the potentially novel species indicated 12 nucleotide differences over a 446 bp region encompassing the V2 and V3 hypervariable regions. From available 16S rRNA gene sequence data, M. leprae-murium shared greatest nucleotide identity with M. avium subsp. paratuberculosis and M. avium. The novel species had a long helix 18 in the V3 region and shared greatest nucleotide identity with M. lepraemurium. The novel species had an additional ‘A’ nucleotide at position 105 of the aligned 16S rRNA gene sequence, the only other mycobacterial database sequence having this same extra nucleotide being M. leprae. This nucleotide variation was exploited to develop specific PCR assays for the two species. These were found to be effective and specific when tested against a panel of mycobacteria including species found in feline leprosy lesions and closely related mycobacteria and also when applied directly to formalin-fixed, paraffin-embedded tissues from feline leprosy cases.—Authors’ Abstract


To analyze the impact on of case finding of leprosy elimination campaigns (LECs), data on newly detected leprosy cases in a leprosy endemic area were collected before, during and after the year of LEC. The number of new leprosy cases detected during the year of LEC was significantly higher than previously. The number of newly detected cases after the year of LEC was similar to
that of detected before the year of LEC in counties with persisting case finding activities. However, the number of newly detected cases after the year of LEC significantly decreased in counties without active case finding activities. The average distance from the homes of leprosy cases detected during LEC to the leprosy control unit at the count town was 62.8 km, which is farther than that of other leprosy cases detected before and after the year of LEC. The average time from disease onset to diagnosis of leprosy cases detected after the year of LEC shortened. The results also showed that carrying out LECs is unlikely to have a significant impact on the trend of case finding within a short time in local areas, but it may improve some indicators of leprosy patients and so promote leprosy control in local areas.—Authors’ Abstract


Though repeated attention has been drawn to a lack of proper teaching-learning modules in leprosy endemic countries, no satisfactory module exists. Keeping in view this fact, we attempted to draft a suitable module on leprosy that could be used to teach leprosy to undergraduate medical students in a simple and comprehensive manner. We used two different modules, Module A and Module B, to teach two different batches of students of the pre-final year (VI and VII semesters) of the MBBS course. Both these modules were conducted by the Department of Dermatology and STD, with participation by the Departments of Microbiology, Pathology and Preventive and Social Medicine. The drafts of the modules were discussed before hand in the Department, keeping in mind the number of days allotted to us. Both the modules were different in certain aspects, but the basic concept was the same. Because Module A had more time, certain practical aspects were also discussed. It was interesting to note that the percentage of increase in the post-test score was 17 for Module A and 15 for Module B, thus proving that both the modules were effective in conveying the core message about leprosy.—Authors’ Abstract


The National Leprosy Eradication Programme (NLEP) is based on survey, education and treatment, including coverage of all the registered cases with multi-drug therapy (MDT). The Government of India introduced MDT in all leprosy endemic districts through a vertical set-up, and through mobile leprosy treatment units in low endemic


OBJECTIVE: To investigate the impact of the current strategy for the elimination of leprosy on its incidence and to assess the consequences of failure to sustain this strategy. METHODS: Scenarios for assessing the impact of the elimination strategy were implemented in a computer simulation program. The scenarios reflected the assumptions made regarding contagiousness, transmission and bacille Calmette-Guerin (BCG) vaccination. The trend in case detection rate for the main countries in which leprosy was endemic during 1985–1998 was fitted, and incidence up to 2020 was projected. FINDINGS: Owing to the gradual shortening of delays in detection up to 1998, and because of the low relapse rate that occurs with multidrug treatment MDT, incidence is predicted to decrease beyond 2000 in all scenarios. The annual decline was a few per cent higher when favourable assumptions were made about protection and coverage of BCG vaccination. Overall, the predicted annual decline in incidences ranged from 2% to 12%. CONCLUSION: The elimination strategy reduces transmission, but the decline may be slow. Relaxation of control after 2005 is unjustified given the uncertainty about the rate of decline and the adverse effects of longer delays in detection. A long-term strategy for leprosy control should be adopted.—Authors’ Abstract
districts. Anti-leprosy work has not been uniform in all the states and needed push-start in some, such as Bihar. There have been spurts of leprosy elimination activities and the entire populations of the regions have not been covered because of various administrative reasons and logistic problems. In Singhbhum district of Bihar, a successful attempt was made to cover the maximum population by campaign approach. The strategy was to involve all the field workers of the leprosy program in the district, supported by a small group of experienced personnel. The campaign, lasting for 39 working days, resulted in detecting leprosy cases equivalent to 64% of cases detected during the previous one full year. The entire operation helped the local staff to gain experience that would be useful for the future of the NLEP, and also provide an insight into working practices. Similar campaign approach can be used in situations where case-detection activities are feeble and the implementation of MDT is slow. If such campaigns are repeated at appropriate intervals, it will be a great support to achieving the goal of leprosy elimination.—Authors’ Abstract


In India there is a dramatic fall in the prevalence rate (PR) of leprosy, but the new case-detection rate (NCDR) has not been reduced concomitantly. It is the operational efficiency of the National Leprosy Eradication Programme (NLEP) that has led to a significant reduction in the NCDR in Andhra Pradesh and Tamil Nadu. The ratio of PR to NCDR has been declining in these two states and it reveals that elimination could be reached even with the high NCDR level of 3 to 4 per 10,000 population, particularly if single skin lesion (SSL) cases are discharged through single dose treatment of rifampicin, ofloxacin and minocycline (ROM). On the other hand, the significant number of cases detected in Bihar and Orissa during modified leprosy elimination campaigns (MLECs) reveals that there are lacunae in operational activities in new case-detection resulting in a large number of undetected cases in the community. Only one-third of the cases are reporting voluntarily. Awareness of leprosy is not adequate to motivate the patients to report voluntarily and complete their treatment, thus underscoring the need for relying on active case-detection so that transmission can be broken and elimination of leprosy achieved. In addition, the influence of socio-economic factors on continued occurrence of leprosy cannot be ruled out. The establishment of a sentinel surveillance system along with a computerized simplified information system to gain in-depth knowledge on the functioning of the NLEP will ensure operational efficiency. In view of this situation, the NLEP should adopt a more realistic approach towards reaching the elimination goal.—Authors’ Abstract


Abstract. Integration of leprosy services into the general health services is regarded as the core strategy to ensure that leprosy control remains cost-effective and equitable, and, thus, sustainable in the coming years. In this article an extensive review is presented of the integration of leprosy services into the general health services. After the rationale of integration is discussed, the article highlights several recent developments within leprosy control and the health sector that are in support of the integration process. An overview is presented of recent experiences in countries that have already embarked on the integration process. Based on these experiences important lessons can be learned and incorporated into a model for the process of integration. This model, which is presented at the end of the article, will assist countries to successfully integrate leprosy services into the general health services.—Cadernos de Saúde Pública

Treatment of Mycobacterium avium disease remains challenging when macrolide resistance develops. We infected C57 beige mice and treated them with mefloquine, SRI-286, and moxifloxacin. SRI-286 (80 mg/kg) was bactericidal in the liver. Mefloquine plus moxifloxacin or mefloquine plus SRI-286 were better than mefloquine alone.—Authors’ Abstract


An important but little recognized side-effect of thalidomide is hypothyroidism.

Three cases of thalidomide-induced hypothyroidism were reported during the early 1960s. In 2002, Badros and colleagues reported a patient who developed severe hypothyroidism about three months after starting thalidomide for multiple myeloma. Furthermore, they found that 14% of patients on thalidomide treatment were subclinically hypothyroid (TSH >10 µ/L) at three months, and suggested that thyroid dysfunction might contribute to some of the known side-effects of the drug, such as fatigue, constipation and bradycardia.—[Abstracted from text] Journal of the Royal Society of Medicine


See Current Literature, Epidemiology and Prevention, p. 556.


Thalidomide has shown to inhibit, selectively and mainly the cytokine tumor necrosis factor-alpha (TNF-alpha), thus, thalidomide has inhibitory consequences on other cytokines; this is ascribed as an immunomodulatory effect. Novel thalidomide analogs are reported with immunomodulatory activity. The aim of this work was to synthesize some of these analogs and to assess them as immunomodulatory agents in an acute model of LPS-induced septic challenge in rat. Animal groups received orally twice a day vehicle carboxymethylcellulose (0.9%), or thalidomide in suspension (100 mg/kg), or analogs in an equimolar dose. Two hours after last dose, rats were injected with saline (NaCl, 0.9%, i.p.) or LPS (5 mg/kg, i.p.). Groups were sacrificed 2 hr after injection and samples of blood and liver were obtained. TNF-alpha, interleukin-6, -1beta, and -10 (IL-6, IL-1beta, IL-10) were quantified by enzyme linked immunosorbent assay (ELISA) and studied in plasma and liver. After 2 hr of LPS-induction, different patterns of measured cytokines were observed with thalidomide analogs administration evidencing their immunomodulatory effects. Interestingly, some analogs decreased significantly plasma and hepatic levels of LPS-induced proinflammatory TNF-alpha and others increased plasma concentration of anti-inflammatory IL-10. Thalidomide analogs also showed slight effects on the remaining proinflammatory cytokines. Differences among immunomodulatory effects of analogs can be related to potency, mechanism of action, and half lives. Thalidomide analogs could be used as a pharmacological tool and in therapeutics in the future.—Authors’ Abstract

Makino, K., Nakajima, T., Shikamura, M., Ito, F., Ando, S., Kochi, C., Ina-

Monodispersed PLGA microspheres containing rifampicin (RFP) have been prepared by solvent evaporation method using a Shirasu porous glass (SPG) membrane. The microspheres were spherical and their average diameter was about 2 microm. The loading efficiency of rifampicin was dependent on the molecular weight of PLGA. The higher loading efficiency was obtained by the usage of PLGA with the lower molecular weight, which may be caused by the interaction of the amino groups of rifampicin with the terminal carboxyl groups of PLGA. PLGA with the monomer compositions of 50/50 and 75/25, of lactic acid/glycolic acid, were used in this study. From rifampicin-loaded PLGA microspheres formulated using PLGA with the molecular weight of 20,000, rifampicin was released with almost constant rate for 20 days after the lag phase was observed for the initial 7 days at pH 7.4. On the other hand, from rifampicin-loaded PLGA microspheres formulated using PLGA with the molecular weight of 5000 or 10,000, almost 90% of rifampicin-loaded in the microspheres was released in the initial 10 days. Highly effective delivery of rifampicin to alveolar macrophages was observed by the usage of rifampicin-loaded PLGA microspheres. Almost 19 times higher concentration of rifampicin was found to be incorporated in alveolar macrophages when rifampicin-loaded PLGA microspheres were added to the cell culture medium than when rifampicin solution was added.—Authors’ Abstract


OBJECTIVE: To evaluate safety and efficacy of thalidomide in the treatment of prurigo nodularis in a group of human immunodeficiency virus (HIV)-infected patients whose condition was recalcitrant to standard treatment. DESIGN: Prospective study. SETTING: Outpatient dermatology and neurology clinic, both referral settings. PATIENTS: Eight HIV-infected patients with refractory prurigo nodularis; a total of 10 met inclusion criteria, but 2 could not be followed up. INTERVENTIONS: Treatment with thalidomide, 100 mg/d. Subjects were randomized after 1 month to receive 100 or 200 mg/d. If side effects were noted, the drug was reduced to a tolerable dose or discontinued. Subjects were monitored at baseline and monthly for degree of pruritus and total area of body involvement of prurigo nodularis. Sequential neurologic assessments were performed. MAIN OUTCOME MEASURES: Efficacy and toxic effects. RESULTS: The dosage of thalidomide ranged from 33 to 200 mg/d. Eight subjects had a greater than 50% response in reduction of itch over 3.4 months (average). Seven subjects had a greater than 50% reduction of skin involvement over 5 months (average). Three subjects developed thalidomide peripheral neuropathy (TPN). There was no correlation between duration of treatment, daily or cumulative dose, and TPN. A change in the Neuropathy Impairment Score of 10 points was a good marker of TPN, as was a greater than 50% decrease in the sural sensory nerve action potential amplitude. CONCLUSIONS: Thalidomide reduced the signs and symptoms of prurigo nodularis in HIV-infected subjects. One third of subjects developed TPN, underscoring the importance of careful neurologic assessment.—Authors’ Abstract


Thalidomide shows moderate inhibitory activity toward neuronal nitric oxide synthase (nNOS) and inducible NOS (iNOS), but not toward endothelial NOS (eNOS). Structural development studies of thalidomide yielded novel phenylhomophthalimide-type NOS in-
Inhibitors with enhanced activity and different subtype selectivity.—Authors’ Abstract


Extracts of the roots of plants of the Geraniaceae family have been used for many years in South Africa as native herbal remedies and there is circumstantial evidence for efficacy in the treatment of pulmonary tuberculosis. We have examined dried roots of Pelargonium reniforme and P. sidoides for antibacterial activity against rapidly growing mycobacteria. Fractions with activity against Mycobacterium aurum and M. smegmatis were obtained from both plant species by bioassay-guided fractionation of n-hexane extracts and were found to contain mixtures of straight-chain fatty acids. Analysis by gas chromatography-mass spectrometry (GC-MS) of the corresponding fatty acid methyl esters revealed structures with chain lengths ranging from C12 to C26. Unsaturated compounds were analyzed as the corresponding dimethyl disulfide adducts to determine double-bond positions. Active mixtures differed in the relative abundance of their components, but all contained 16:0 (palmitic), Delta9-18:1 (oleic) and Delta9,12-18:2 (linoleic acid) as the major components. When tested against M. aurum, M. smegmatis and other rapidly growing mycobacteria (M. fortuitum, M. abscessus and M. phlei), all saturated compounds except 12:0 were devoid of antimycobacterial activity, whereas unsaturated compounds showed antimycobacterial activity related to their degree of unsaturation, their chain length and the bacterial species tested. The most potent compound was linoleic acid, with MIC of 2 mg/l against M. aurum.—Authors’ Abstract

Shimizu, S., Suzuki, M., Tomoda, A., Arai, S., Taguchi, H., Hanawa, T., and Kamiya, S. Phenoxazine compounds produced by the reaction of o-aminophenol or its derivatives with bovine hemoglobin, on seven species of mycobacteria such as Mycobacterium tuberculosis, Mycobacterium marinum, Mycobacterium intracellulare, Mycobacterium scrofulaceum, Mycobacterium fortuitum, Mycobacterium kansaii and Mycobacterium smegmatis and some bacteria such as Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica serovar Typhimurium, Staphylococcus aureus, Listeria monocytogenes. These phenoxazines, including 2-amino-4, 4alpha-dihydro-4alpha, 7-dimethyl-3H-phenoxazine-3-one (Phx-1), 3-amino-1, 4alpha-dihydro-4alpha, 8-dimethyl-2H-phenoxazine-2-one (Phx-2), and 2-amino phenoxazine-3-one (Phx-3), prevented the proliferation of four non-tuberculosis mycobacteria including M. scrofulaceum, M. kansaii, M. marinum, and M. intracellulare dose-dependently, though the inhibitory effects of these phenoxazines differed according to the species of mycobacteria. However these phenoxazines failed to prevent the proliferation of M. tuberculosis, M. fortuitum, and M. smegmatis, and the concerned bacteria other than mycobacteria. The present results may contribute to development of novel antibiotics against non-tuberculosis mycobacteria.—Authors’ Abstract


It has been shown that some antibiotics can modify cytokine production. We have examined the effect of rifampicin on secretion of interleukin-1beta (IL-1beta), IL-6, IL-10, and tumor necrosis factor alpha (TNF-alpha) by lipopolysaccharide (LPS)-stimulated or heat killed staphylococci (Pansorbin) stimulated monocytes. Secretion of IL-1beta and TNF-a were significantly inhibited (p <0.002) whereas secretion of IL-6 and IL-10 were significantly increased (p <0.003) by rifampicin treated mononuclear cells. Rifampicin had immunomodulatory effects through its capacity to alter the secretion of tested cytokines by human monocytes.—Authors’ Abstract
Leprosy neuropathy is characterized by initial involvement of the small nerve fibers, later followed by involvement of the large fibers, when routine nerve conduction studies become abnormal. To increase the diagnostic yield and precocity of these studies, we applied the near nerve technique to the sural nerve of 8 leprosy patients. Contrary to our expectations, the main component of the sural nerve sensory action potential was abnormal in all patients, but the minimum conduction velocity originating from small 3–6 mm fibers was normal or only mildly involved in three patients. Also, although Schwann cells are the first to be involved in leprosy, the results are suggestive of axonal degeneration instead of demyelination. To better understand the neurophysiology and physiology of leprosy and to increase the accuracy and precocity of the diagnosis, it will be necessary to investigate patients in the very early stages of the disease and to correlate these findings with the corresponding nerve pathology.—Authors’ Abstract


OBJECTIVE: To evaluate and compare two strengths of topical phenytoin sodium suspension (2% and 4%) with normal saline in the healing of acute trophic ulcers in leprosy patients. METHODS: A prospective, parallel, double-blind, randomized study was conducted in 45 leprosy inpatients with acute trophic ulcers. Patients were randomized to receive 2%, 4% or normal saline dressing on their ulcers once daily for 4 weeks. Efficacy parameters such as a reduction in the surface area of the ulcer, bacterial culture of the ulcer swab, appearance of healthy granulation tissue, cessation of ulcer discharge and overall gradation of clinical healing and safety were assessed at weekly intervals. RESULTS: The ulcer area reduction was greater in the 2% and 4% phenytoin groups compared with the normal saline group (p <0.001). Appearance of healthy granulation tissue and cessation of ulcer discharge was also observed earlier in the two phenytoin groups. At the end of 4 weeks, 11 ulcers each had healed completely in both the 2% and 4% phenytoin groups compared with none in the control group. There were no statistical differences between the 2% and 4% phenytoin groups. No side effects were reported by any patient. CONCLUSION: Topical phenytoin appears to be an effective, safe and cheap therapeutic option for the healing of trophic ulcers in leprosy patients.—Authors’ Abstract


Background: Leprosy is a rare but serious mycobacterial infection. Immigration from areas where the disease is endemic has resulted in the importation of leprosy into countries where it is not endemic and where physicians and health care workers have little or no experience in diagnosis and therapy. In this study we characterized leprosy patients seen in a tropical disease unit that manages most of the reported leprosy cases in Canada. Methods: We reviewed the clinical records of all 184 leprosy patients who were referred to the tropical Disease Unit at Toronto General Hospital, Toronto, Ontario, Canada, between 1979 and 2002 and abstracted demographic and clinical information. Results: Patients were more likely to be male (122 or 66.3%) and of Indian (44 or 23.9%), Filipino (49 or 22.6%) or Vietnamese (37 or 20.1%) origin. Patients experienced symptoms for a mean of 4.8 yrs before referral to the Tropical Disease Unit.
Most had no family history of leprosy (152/172 or 84%). Most patients presented either with borderline tuberculoid (80 or 43.5%) or borderline lepromatous (37 or 20.1%) disease. On average, patients presented with 5.8 skin lesions. Upper- and lower-extremity nerve dysfunction was common at presentation, with up to one-third of patients demonstrating either sense or motor loss. A significantly greater lag time to presentation was observed in patients who emigrated from low-prevalence regions (p < 0.001). Interpretation: Leprosy is a chronic infectious disease that is associated with serious morbidity if left untreated. Leprosy is uncommon in developed countries, but it is important for physicians to have a high index of suspicion when a foreign-born patient presents with chronic dermatitis and peripheral nerve involvement.—Tropical Disease Bulletin


A hospital-based retrospective study on childhood leprosy was carried out at B.R. Koirala Institute of Health Sciences, Dharan, covering the period April 1998–April 2002. 20 (4.45%) leprosy patients were detected in children aged 6–14 years. The male:female ratio was 4:1. History of contact was found in 10% of the patients. The commonest type of leprosy was borderline tuberculoid leprosy (550%), followed by borderline lepromatous leprosy (301%). Most of the patients had more than one lesion. Nerve involvement and grade 2 deformity were noted in 55% and 20% of the patients, respectively. Slit skin smear was positive in 30% of patients.—Authors’ Abstract


Leprosy among children is a public health problem reflecting the disease’s transmission in the community and the efficiency of control programs. To evaluate some clinical, epidemiological and histopathological criteria, as well as the level of agreement between clinical and histopathological diagnoses, 207 biopsies were studied from patients less than 15 years old who were clinically diagnosed with leprosy between March 1994 and September 2000. Leprosy was confirmed by histopathology in 119 cases (57.5 per cent). Forty-seven per cent of children were 10 years old or more; 28.5 per cent shared their dwellings with leprosy patients; 35 per cent had only one lesion, and 43 per cent were multibacillary cases. Agreement between clinical and histopathological classification was 36 per cent; hypochromic chronic eczema and post-inflammatory incontinence of melanin pigment were the clinical lesions most frequently mistaken with leprosy. Leprosy among children represents 7 per cent of new leprosy cases in Colombia and the high percentage of multibacillary cases suggests that diagnosis is being made late. The disease must be investigated in all children living with leprosy patients and skin biopsy is recommended to avoid false-positive diagnoses.—Tropical Disease Bulletin


Background: Immune reconstitution inflammatory syndrome (IRIS) is an unusual inflammatory reaction to an opportunistic infection that occurs in human immunodeficiency virus (HIV)-positive patients with profound immunosuppression during the reconstitution of the immune system in the initial months of highly active antiretroviral treatment.

Observations: We describe 3 cases of leprosy occurring in patients treated with a combination of 3 antiretroviral drugs who fulfilled the criteria for IRIS. A reactional state occurred in all 3 cases. Two of the 3 patients presented an unusual ulcerous progression of the lesions not generally observed in cases of leprosy. The outcome was
favorable in all 3 cases. The frequency of IRIS associated with leprosy in French Guiana and Martinique is estimated at 3 cases per 1000 HIV-positive patients receiving highly active antiretroviral treatment.

**Conclusions:** Leprosy should be recognized as an IRIS-associated infection with possibility of atypical presentation.—Archives of Dermatology


A 30-year-old man presented to the Hansen outpatient department with swelling and ulceration of toes for 2 months and swelling of the right fifth and fourth fingers and the left second finger for 1 month. In addition to skin lesions of lepromatous leprosy (subpolar type), there was nontender, nonfluctuant swelling of the right fifth and fourth fingers and left second finger. Skin over the right fifth finger showed sinus-like openings with associated purulent discharge. He also had swelling and ulceration of second left toe. Slit-skin smear (SSS) showed a bacterial index of 6+ from the ear lobes and cutaneous nodules, 4+ from the patch, and 3+ from normal skin. Modified Ziehl-Neelsen staining of the discharge extruding from the sinuses on the right fifth finger also showed abundant acid-fast bacilli. Radiography of the hands and feet showed lytic lesions in the distal epimeta physeal region of proximal phalanx of the right fifth finger and left second finger and erosion of distal end of proximal phalanges of both second toes. Histopathological examination of biopsy specimen from the patch (back) showed features of lepromatous leprosy, and Fite-Faraco stain for tissue acid-fast bacteria (AFB) was strongly positive. Fine-needle-aspiration cytology (FNAC) from the lytic lesion in the bone also showed predominantly foamy macrophages with strongly positive staining for AFB with a few interspersed lymphocytes, epithelioid cells and Langhans giant cells. On the basis of these features, a clinical diagnosis of subpolar lepromatous leprosy with leprous osteitis was made. In today’s clinical era of improved case detection and prompt treatment with effective multidrug regimens, advanced bone changes are rarely encountered. We describe this case of lepromatous leprosy that developed cavitating lesions of the phalanges of the hand, seen on x-ray as well-defined bone cyst and erosions.—Authors’ Abstract


We report a case of borderline tuberculoid leprosy complicated by a median nerve abscess, acute renal failure secondary to rifampicin-induced haemolysis and duodenal ulceration secondary to steroid use. Rifampicin induced haemolysis is a rare and probably under-reported complication of leprosy multi-drug therapy. It should be considered when patients complain of flu-like symptoms after taking their monthly rifampicin.—Authors’ Abstract


Patients with leprosy may have only nerve involvement without skin changes. These cases are known as pure neural leprosy and can be seen in 10% of leprosy patients. Most patients have mononeuritic or multiple mononeuritic neuropathy patterns. The isolated lesion of the superficial peroneal nerve is uncommonly seen. We report a patient with involvement of this nerve in which there was no thickening of superficial nerves. The performed nerve biopsy showed inflammatory infiltration, loss of fibers and presence of *Mycobacterium leprae*. We believe that in prevalent leprosy countries we should take in mind the possibility of isolated pure neural leprosy in some patients without skin lesion. In these cases the diagnosis of leprosy is impossible on clinical
grounds and nerve biopsy is mandatory.—
Authors’ Abstract

**Helmer, K. A., Fleischfresser, I.,

**Summary:** The Lucio’s phenomenon, a type 2 reactional condition in leprosy probably mediated by immune complexes, is a severe necrotizing skin reaction that occurs mainly in patients with non-nodular lepromatous leprosy. This report presents a 27-year-old woman, in her 32nd week of pregnancy, with a one-week history of painful skin lesions in extremities, reddish-purple, sharply delineated, confluent, with bullae and occasional necrosis and ulceration. The patient also referred fever. Bacilloscopy showed acid-fast bacilli and globi, and the histopathologic findings of a skin biopsy were consistent with lepromatous leprosy and Lucio’s phenomenon. Prednisone and multidrug therapy with rifampin, clofazimine and dapsone were given, with remission. Pregnancy has been associated with a high incidence of first diagnosis of leprosy or with an exacerbation of symptoms in patients with the established disease because hormonal alterations cause immunological imbalance, particularly between the last three months of pregnancy and the first three months of lactation, when immunosuppression is higher. Despite the recommendation not to take drugs during pregnancy, the multidrug therapy regimen must be used, since the benefits achieved with the treatment surpass the risks.—Anais Brasileiros de Dermatologia


Nerve involvement is common to the pathogenesis of both leprosy and herpes zoster. We report two cases of borderline leprosy in which the skin lesions characteristically spared the healed zoster scar. Possible mechanisms and relationship are discussed.—Authors’ Abstract


The objectives of our study were to describe and analyze the malignancies that occurred in plantar ulcers of leprosy patients. The possible predisposing conditions, duration and extent of the spread of the tumour were also studied. All patients with trophic ulcer of the foot attending the urban leprosy clinic in our hospital from January 1998 to January 2003 were screened for change to malignancy. During the study period, 79 cases of plantar ulcers in leprosy were seen. The mean age of these cases was 39.9 years with male-to-female ratio of 4:1. Eleven cases with plantar ulcers and malignant change were diagnosed in our hospital during the study period. The male-to-female ratio was 4.5:1. The mean age of these patients was 60.6 years. Their age ranged from 46 to 75 years. Nine of the cases were treated cases of borderline tuberculoid leprosy, while two had treated lepromatous leprosy. In our study, two distinct morphological types of malignant changes were seen. Histopathologically, all cases, except one, were of well-differentiated squamous cell carcinoma variation; one case had verrucous carcinoma. Though trophic ulcers are common in leprosy cases, only long-standing and neglected ones undergo malignancy.—Authors’ Abstract


This study reports the follow-up results of 36 highly bacillated untreated BL/LL cases who were serially allocated to three treat-
ment groups. Group I patients received a modified WHO regimen (Rifampicin 600 mg once a month supervised, 50 mg of Clofazimine and 100 mg of Dapsone daily unsupervised) and BCG 0.1 mg per dose 6 monthly; group II patients received the same multi-drug treatment (MDT) and Mw (2 × 10⁸ killed bacilli per dose) 6 monthly; group III patients received the same MDT with 0.1 ml of distilled water 6 monthly and acted as a control. Treatment was continued till smear negativity. All these three groups were comparable by their initial clinical score, bacteriological index (BI), viable bacilli as assessed by the mouse footpad (MFP), bacillary adenosine triphosphate (ATP) content and also histologically at the time of starting treatment. All these parameters were evaluated every 6 months. The vaccines were well tolerated. All the patients in group I became smear negative by 3.5 years, in group II in 3 years whereas those in group III took 5 years. The incidence of reactions was the same in all the groups during the first 2 years, however, patients of group III (MDT + placebo) continued to have reactions up to 3 years. No viable bacilli could be detected in the local and distal sites as estimated by MFP and bacillary ATP after 12 months in both the immunotherapy groups. These could be detected in patients on MDT alone up to 24 months of therapy. Histologically patients in both the immunotherapy groups (groups I and II) showed accelerated granuloma clearance, histological upgrading and non-specific healing without granuloma formation both at the local and distal sites and this was achieved much earlier compared to the MDT + placebo group. Thus, by the addition of immunotherapy the effective treatment period of achieving bacteriological negativity could be reduced by about 40%, time period of reactions reduced by 33% and there were no reactions and/or relapses in the 10–12 years post-treatment follow-up. — Authors’ Abstract


BACKGROUND: Leprosy or Hansen’s disease (HAD) undoubtedly remains an emergency in certain countries. It is an ancient deforming disease caused by Mycobacterium leprae. The countries with the highest endemic leprosy rate in 2000 were Brazil, India and Madagascar. In Italy, the old epidemic has been defeated and there are approximately 400 patients under constant monitoring with three to four new cases per year involving Italian residents. The kidney is one of the target organs during the splanchnic localization of leprosy. The histopathological renal lesion spectrum includes glomerulonephritis (GN), renal amyloidosis (RA) and interstitial nephritis (IN). Both proteinuria and chronic renal failure are the main clinical expressions of renal damage in leprosy. To the best of our knowledge, very little is reported concerning end-stage renal disease (ESRD) in leprosy patients both in the most important national and international renal registries and in the available literature. This study aimed to report the long-term experience of our department in this field. METHODS: To achieve this, we analyzed retrospectively the HAD Center (Gioia del Colle) database at our hospital. RESULTS: Eight leprosy patients were dialyzed from 1980 to June 2003 (six males and two females), with a mean age of 61.0 ± 8.9 S.D. yrs (range: 51–76) and a mean HAD duration of 36.1 ± 5.1 yrs. The first clinical nephropathy manifestations were non-nephrotic proteinuria associated with chronic renal failure in four patients, and nephrotic proteinuria in four patients. Kidney biopsies performed in three patients showed two had RA, and one had IN. Two patients were treated initially by peritoneal dialysis; they were then switched to hemodialysis (HD) after 3 and 10 months because of recurrent peritonitis. HD treatment lasted 40.6 ± 31.4 months (range: 9–101). Six patients died, one due to hyperkalemia, one because of a technical dialysis accident, and the remainder due to causes unrelated to the dialysis treatment. Two patients are still alive, treated with HD for 17 and 44 months. CONCLUSIONS: Uremia represents a late complication of leprosy and has a multifactorial genesis, although RA is among the most frequent causes, conventional bicarbonate HD appears to offer good results in
the treatment of uremia in leprosy patients.—Authors’ Abstract


The present paper reviews the anatomy of palmaris longus muscle and also the situations where palmaris longus muscle has been used as an independent motor or as a donor of tendon graft material. Its relevance in leprosy-affected hands is also discussed because the muscle is usually spared in hand palsies consequent to leprotic neural damage. The advantages and disadvantages of its use in different operative procedures have been analyzed. The author’s experience with this muscle in the correction of hand deformities in leprosy is described.—Author’s Abstract


Leprosy is one of the most common causes of nontraumatic peripheral neuropathy in the developing world. The causative agent, *Mycobacterium leprae*, has a predilection for Schwann cells, where the organism multiplies unimpeded by organism-specific host immunity, resulting in destruction of myelin, secondary inflammatory changes, and destruction of the nerve architecture. The cardinal diagnostic features of leprosy are anesthetic skin lesions, neuropathy, and positive skin smears for the bacilli. However, patients may rarely present without skin lesions in pure neuritic leprosy. Electrodiagnostic findings early in the disease reveal demyelinating features, such as slowing of conduction velocity and prolongation of latencies, but as the disease progresses secondary axonal damage commonly ensues. Electrodiagnostic studies are also useful to monitor for toxicity secondary to therapy, particularly thalidomide-associated neuropathy. Nerve biopsy of a sensory cutaneous nerve is sometimes essential to confirm a diagnosis of leprosy. Significant advances in understanding of the pathogenesis, mapping of the genome, and other advances in molecular biology may result in better preventive and therapeutic modalities, and the goal of eradicating leprosy as a global problem may yet be realized.—Authors’ Abstract


We report a case with abdominal complications of clofazimine treatment which included blackish discolouration of the lymph nodes, omentum and peritoneum. A 44-year-old female with lepromatous leprosy and a history of adverse reaction to clofazimine 2 years previously, presented with rectosigmoid junction adenocarcinoma. Laparotomy revealed an inoperable tumour with pigmentation of the bowel, serosa and peritoneum. A second operation had to be performed for transverse loop colostomy and a mesenteric lymph node biopsy sent for frozen section showed typical clofazimine crystals. Despite widespread use for many years in the treatment of leprosy, this drug is not known to be carcinogenic and this case provides no evidence for an association or link between its use and the patient’s cancer. Apart from its use in leprosy, clofazimine may be used in the treatment of disseminated *Mycobacterium avium*-intracellulare infection, Buruli ulcer due to *M. ulcerans* and occasionally in other mycobacterial infections. An awareness of the rare side-effect described above may help in the clinical assessment and management of such cases, including the avoidance of unnecessary laparotomy.—Authors’ Abstract

This is a retrospective cohort study of 103 multibacillary leprosy patients (18% BB, 48% BL and 34% LL) followed during and after treatment, in a tertiary referral centre with an outpatient clinic in an endemic area in Brazil, for an average period of 65 months since the start of multidrug therapy (24-dose MDT). The objective of the study was to identify the role of overt neuritis (presence of pain in a peripheral nerve trunk, with or without enlargement or neural function damage), in the development of impairments. They were evaluated using the World Health Organization disability grade before treatment, at the end of the treatment, and at the end of the follow-up period. Thirty-four percent of patients presented overt neuritis during MDT, and 45% had overt neuritis episodes during the follow-up period; the most commonly affected nerves were ulnar, fibular and posterior tibial nerves, and the neuritic episodes were carefully treated with steroid therapy and physiotherapy. Impairments were associated with: affected (painful and/or thick) nerves at diagnosis (p <0.005); delay in diagnosis (p = 0.010); impairments already present at the start of treatment (p = 0.00041 at the end of MDT, and p = 0.000013 at the end of follow-up); occurrence of overt neuritis episodes during MDT (p = 0.0016) or the whole follow-up (p = 0.015). These data draw attention to the importance of early diagnosis and of good neurological examination throughout the follow-up, as well as suggest the importance of neuritis in the induction of impairments in multibacillary leprosy.—Authors’ Abstract


Reactions in leprosy causing nerve function impairment (NFI) are increasingly treated with standardized regimens of corticosteroids, often under field conditions. Safety concerns led to an assessment of adverse events of corticosteroids, based on data of three trials studying prevention of NFI (the TRIPOD study). A multicenter, randomized, double-blind placebo-controlled trial was conducted in leprosy control programs in Nepal and Bangladesh. Treatment was with prednisolone according to fixed schedules for 16 weeks, starting in one trial with 20 mg/day (prophylactic regimen: total dosage 1.96 g) and in the other two trials with 40 mg/day (therapeutic regimen: total dosage 2.52 g). Minor adverse events were defined as moon face, fungal infections, acne, and gastric pain requiring antacid. Major adverse events were defined as psychosis, peptic ulcer, glaucoma, cataract, diabetes and hypertension. Also, the occurrence of infected plantar, palmar, and corneal ulceration was monitored, together with occurrence of TB. Considering all three trials together, minor adverse events were observed in 130/815 patients (16%). Of these, 51/414 (12%) were in the placebo group and 79/401 (20%) in the prednisolone group. The relative risk for minor adverse events in the prednisolone group was 1.6 (p = 0.004). Adverse events with a significantly increased were acne, fungal infections and gastric pain. Major adverse events were observed in 15/815 patients (2%); 7/414 (2%) in the placebo group and 8/401 (2%) in the prednisolone group. No major adverse events had a significantly increased risk in
the prednisolone arm of the trials. No cases of TB were observed in 300 patients who could be followed-up for 24 months. Standardized regimens of corticosteroids for both prophylaxis and treatment of reactions and NFI in leprosy under field conditions in developing countries are safe when a standard pre-treatment examination is performed, treatment for minor conditions can be carried out by field staff, referral for specialized medical care is possible, and sufficient follow-up is done during and after treatment.—Tropical Disease Bulletin


A patient with lepromatous leprosy, while on WHO multidrug therapy (MDT) for multibacillary disease, was diagnosed as having dapsone syndrome with recurrent episodes of bullous lesions on the lower extremities for 4–5 years. The lesions were associated with high-grade fever. Examination revealed multiple hypopigmented macules on the limbs. Multiple atrophic scars were also found on the buttocks and lower limbs. Bilateral ulnar, radial cutaneous and lateral popliteal nerves were thickened. On day 10 of WHO-MB-MDT he developed a flaccid bulla on the lower leg. Skin slit smear showed a bacterial index (BI) of 3+ and the histopathology was consistent with type II reaction. High dose corticosteroid therapy was started but he continued to have new lesions, and was therefore referred to a centre where thalidomide was available. Clinical response was good and he remained symptom-free after gradual reduction in dosage. ENL should be differentiated from bullous drug reactions, pemphigus vulgaris, bullous pemphigoid and other blistering diseases.—Authors’ Abstract


OBJECTIVE: Motor and sensory nerve conductions, F responses, sympathetic skin responses and R-R interval variations (RRIV) were studied to determine the type of peripheral neuropathy among patients with leprosy. METHODS: Twenty-nine consecutive patients with leprosy (25 male, 4 female) hospitalized in the “Istanbul Leprosy Hospital” between January–December, 1999 were included in this study. Ten patients had borderline lepromatous leprosy, and 19 had lepromatous leprosy. None of the patients studied had the tuberculoid form. The mean age was 55 ± 12 years. The control group consisted of 30 (26 male, 4 fe-
male) healthy volunteers (mean age: 58.1 ± 7.8 years). All subjects included in the study underwent neurological examination and electrophysiological evaluation. Standard procedures were performed for evaluating sensory and motor conduction studies. Motor studies were carried out on both left and right median, ulnar, tibial and common peroneal nerves while median, ulnar, sural and superficial peroneal nerves were examined for sensory studies. Sympathetic skin response recordings on both hands and RRIV recordings on precordial region were done in order to evaluate the autonomic involvement. RESULTS: The lower extremity was found to be more severely affected than the upper, and sensory impairment predominated over motor. Of 58 upper limbs examined, no sympathetic skin responses was recorded in 46 (79.3%). Compared with the controls, the RRIVs of the leprosy patients were found to be reduced during both resting and deep forced hyperventilation. CONCLUSION: Our results indicate that leprosy causes a predominantly axonal polyneuropathy that is more severe in the lower extremities. Sensory nerve damage is accompanied by autonomic involvement.—Authors’ Abstract


PURPOSE: The extensor to flexor 4-tailed tendon transfer (EF4T) and the palmaris longus 4-tailed tendon transfer (PL4T) are 2 surgical procedures used to correct intrinsic paralysis of the hand in leprosy. The EF4T traditionally is the more common procedure and requires the transfer of a wrist extensor muscle. The PL4T requires the transfer of the palmaris longus and morbidity is expected to be lower. A follow-up study was performed to determine whether the clinical outcome of the PL4T is superior to the EF4T procedure in leprosy patients with ulnar claw fingers that are considered mobile before surgery. METHODS: Fifty-five patients presented 65 affected hands, of which 40 hands had the PL4T and 25 had the EF4T procedure. Each hand was assessed before surgery and at follow-up evaluation by predetermined angle measurements, standardized photographs, mechanical function, and patient satisfaction. Each hand was given an overall technical grade according to previously published standards. RESULTS: After an average follow-up period of 33 months there was no statistically significant difference in the technical outcome or patient satisfaction between the 2 tendon transfer procedures. CONCLUSIONS: Whenever the palmaris longus is available it may be considered to be the motor tendon of choice to undertake a many-tailed procedure for claw finger reconstruction in mobile hands paralyzed by leprosy. The palmaris longus should be considered as a possible motor tendon when correcting intrinsic muscle paralysis of the hand.—Authors’ Abstract


This study was designed to investigate whether leprosy patients diagnosed with mild sensory impairment have a better prognosis when treated with steroids than similarly impaired patients treated with placebo. A multicenter, randomized, double-blind, placebo-controlled trial was conducted in Nepal and Bangladesh [date not given]. Patients were eligible if they had a confirmed leprosy diagnosis, were between 15 and 50 years old, had mild sensory impairment of the ulnar or posterior tibial nerve of less than 6 months duration and did not require steroids for other reasons. ‘Mild impairment’ was defined as ‘impaired on the Semmes-Weinstein monofilament test, but testing normal on the balpen sensory test.’ Subjects were randomized to either prednisolone treatment starting at 40 mg per day, tapering over 4 months, or placebo. Nerve function was monitored monthly. Any patient who deteriorated was taken out of the trial and was put on full-dose steroid treatment. Outcome assessment was done at 4, 6,
9 and 12 months from the start of the treatment. Outcome measures were the proportion of patients needing full-dose prednisolone and the Semmes-Wenmstein sum scores. Each patient contributed only one nerve to the analysis. Seventy-five patients had nerves eligible for analysis, of whom 41 (55%) and 34 (45%) were allocated to the prednisolone and placebo arms, respectively. At 4 months, three patients in the prednisolone arm (7%) and six in the placebo arm (18%) had an outcome event requiring full-dose steroids. At 12 months, these proportions had almost reversed, 11 (27%) and 6 (18%) in the treatment and placebo arms, respectively. In the latter group, 75% had recovered spontaneously after 12 months. Prednisolone treatment of sensory impairment of the ulnar and posterior tibial nerves detectable with the monofilament test, but not with the balloon test, did not improve the long-term outcome in terms of recovery of touch sensibility, nor did it reduce the risk of leprosy reactions or nerve function impairment beyond the initial 4-month treatment phase. Two unexpected main findings were the strong tendency of mild sensory impairment to recover spontaneously and the fact that patients with mild sensory impairment without any other signs or symptoms of reaction or nerve function impairment are relatively rare.—Tropical Disease Bulletin

**Immunopathology**


Using a short-term bulk culture protocol designed for an intracellular-staining method based on a flow cytometry approach to the frequencies of cytokine-producing cells from tuberculosis and leprosy patients, we found distinct patterns of T cell subset expression. The method also reveals the profile of peak cytokine production and can provide simultaneous information about the phenotype of cytokine-producing cells, providing a reliable assay for monitoring the immunity of these patients. The immune response of *Mycobacterium leprae* and purified protein derivative (PPD) in vitro to a panel of mycobacteria-infected patients from an endemic area was assessed in primary mononuclear cell cultures. The kinetics and source of the cytokine pattern were measured at the single-cell level. IFN-gamma-, TNF-alpha-, IL-4- and IL-10-secreting T cells were intracytoplasmic evaluated in an attempt to identify *M. leprae* and PPD-specific cells directly from the peripheral blood. The analysis by this approach indicated that TNF-alpha was the first (8 hr) to be produced, followed by IFN-gamma (16 hr), IL-10 (20 hr) and IL-4 (24 hr), and double-staining experiments confirmed that CD4+ were a greater source of TNF-alpha than of CD8+ T cells (p <0.05). Both T cell subsets secreted similar amounts of IFN-gamma. We conclude that the protocol permits rapid evaluation of cytokine production by different T cell populations. The method can also be used to define immune status in non-infected and contact individuals.—Authors’ Abstract


*Mycobacterium avium* uptake by human macrophages differs between the phenotypes of bacterium grown in laboratory media (extracellular growth, EG) and bacterium grown within macrophages (intracellular growth, IG). Studies *in vivo* have confirmed that, when spreading, pathogenic mycobacteria enter macrophages by a complement receptor 3-independent pathway, in contrast to mycobacteria uptake *in vitro*. *M. avium*, grown in macrophages (IG) for 3 or more days, invade fresh macrophages by a macroinocytosis-
like mechanism, in contrast to bacteria grown in media (EG), confirmed by the inhibitory effect of wortmannin, an inhibitor of phosphoinoside-3-kinase, on the uptake of IG, but not EG, by macrophages. The IG phenotype was seen in vacuoles with lower pH than those inhabited by the EG phenotype. Incubation of macrophages with bafilomycin A1, an inhibitor of vacuole acidification, decreased the viability of intracellular IG, but not EG, phenotype, suggesting the importance of an acidic environment for the regulation of IG genes. In addition, the percentage of vacuoles that incorporate and retain LAMP-1 is smaller with EG than with IG bacteria. The formation of *M. avium* macropinosomes was also shown to be independent of microtubules. These data suggest that uptake of extracellular fluid is part of *M. avium* IG phenotype uptake by macrophages, and that the IG phenotype inhabits a slightly different vacuole than that of EG.—Authors’ Abstract


A central paradox of tuberculosis immunity is that reinfection and bacterial persistence occur despite vigorous host immune responses concentrated in granulomas, which are organized structures that form in response to infection. Prevailing models attribute reinfection and persistence to bacterial avoidance of host immunity via establishment of infection outside primary granulomas. Alternatively, persistence is attributed to a gradual bacterial adaptation to evolving host immune responses. We show here that superinfecting *Mycobacterium marinum* traffic rapidly into preexisting granulomas, including their caseous (necrotic) centers, through specific mycobacterium-directed and host cell-mediated processes, yet adapt quickly to persist long term therein. These findings demonstrate a failure of established granulomas, concentrated foci of activated macrophages and antigen-specific immune effector cells, to eradicate newly deposited mycobacteria not previously exposed to host responses.—Authors’ Abstract


The 65-kDa mycobacterial heat shock protein (Hbsp65) has been invoked in the pathogenesis of both adjuvant arthritis (AA) in the Lewis rat (RT.1(l)) and human rheumatoid arthritis. Arthritic Lewis rats in the late phase of AA show diversification of the T cell response to Bctd C-terminal determinants (Ctcd), and pretreatment of naive Lewis rats with a mixture of peptides representing these neoepitopes affords protection against AA. However, the fine specificity and physiologic significance of the Ctcd-directed T cell repertoire, and the role of homologous self (rat) hsp65 (Rhsp65), if any, in spreading of the T cell response to Bctd have not yet been examined. We observed that T cells primed by peptides comprising Ctcd can adoptively transfer protection against AA to the recipient Lewis rats. However, these T cells can be activated by preprocessed (peptide) form of Ctcd, but not native Bctd, showing that Ctcd are cryptic epitopes. The Ctcd-reactive T cells can be activated by the naturally generated (dominant) C-terminal epitopes of both exogenous and endogenous Rhsp65 and vice versa. Furthermore, certain individual peptides constituting Ctcd and their self homologs can also induce protection against AA. These results support a model for the diversification of T cell response to Bctd during the course of AA involving up-regulation of the display of cryptic Ctcd coupled with spontaneous induction of T cell response to the cross-reactive dominant C-terminal epitopes of Rhsp65. The identification of disease-regulating cryptic determinants in Ags implicated in arthritis provides a novel approach for immunotherapy of rheumatoid arthritis.—Authors’ Abstract
A group of T cells recognizes glycolipids presented by molecules of the CD1 family. The CD1d-restricted natural killer T cells (NKT cells) are primarily considered to be self-reactive. By employing CD1d-binding and T cell assays, the following structural parameters for presentation by CD1d were defined for a number of mycobacterial and mammalian lipids: two acyl chains facilitated binding, and a polar head group was essential for T cell recognition. Of the mycobacterial lipids tested, only a phosphatidylinositol mannoside (PIM) fulfilled the requirements for CD1d binding and NKT cell stimulation. This PIM activated human and murine NKT cells via CD1d, thereby triggering antigen-specific IFN-gamma production and cell-mediated cytotoxicity, and PIM-loaded CD1d tetramers identified a subpopulation of murine and human NKT cells. This phospholipid, therefore, represents a mycobacterial antigen recognized by T cells in the context of CD1d.—Authors’ Abstract

Collectins, including surfactant proteins A (SP-A) and D (SP-D) and mannose binding lectin (MBL), are the important constituents of the innate immune system. *Mycobacterium avium*, a facultative intracellular pathogen, has developed numerous mechanisms for entering mononuclear phagocytes. In this study, we investigated the interactions of collectins with *M. avium* and the effects of these lectins on phagocytosis of *M. avium* by macrophages. SP-A, SP-D, and MBL exhibited a concentration-dependent binding to *M. avium*. The binding of SP-A to *M. avium* was Ca(2+)-dependent but that of SP-D and MBL was Ca(2+)-independent. SP-A and SP-D but not MBL enhanced the phagocytosis of FITC-labeled *M. avium* by rat alveolar macrophages and human monocyte-derived macrophages. Excess mannan, zymosan, and lipoarabinomannan derived from the *M. avium*-intracellular complex, significantly decreased the collectin-stimulated phagocytosis of *M. avium*. Enhanced phagocytosis was not affected by the presence of cycloheximide or chelation of Ca(2+). The mutated collectin, SP-A(E195Q, R197D) exhibited decreased binding to *M. avium* but stimulated phagocytosis to a level comparable to wild-type SP-A. Enhanced phagocytosis by cells persisted even after preconditioning and removal of SP-A or SP-D. Rat alveolar macrophages that had been incubated with SP-A or SP-D also exhibited enhanced uptake of (125)I-mannosylated BSA. Analysis by confocal microscopy and flow cytometry revealed that the lung collectins up-regulated the cell surface expression of mannose receptor on monocyte-derived macrophages. These results provide compelling evidence that SP-A and SP-D enhance mannose receptor-mediated phagocytosis of *M. avium* by macrophages.—Authors’ Abstract

Interleukin-18 (IL-18) has been demonstrated to synergize with BCG for induction of a T-helper-type 1 (Th1) immune response. Since successful treatment of superficial bladder cancer with BCG requires proper induction of Th1 immunity, we have developed a recombinant (r) BCG strain that functionally secretes mouse (m) IL-18. This rBCG-mIL-18 strain significantly increased...
production of the major Th1 cytokine IFN-gamma in splenocyte cultures, at levels comparable to that elicited by control BCG plus exogenous rIL-18. IFN-gamma production by splenocytes was eliminated by addition of neutralizing anti-IL-18 antibody. Endogenous IL-12 played a favorable role whereas IL-10 played an adverse role in rBCG-mIL-18-induced IFN-gamma production. Enhanced host antimycobacterial immunity was observed in mice infected with rBCG-mIL-18 which showed less splenic enlargement and reduced bacterial load compared to control mice infected with BCG. Further, splenocytes from rBCG-mIL-18-infected mice, in response to BCG antigen, displayed increased production of IFN-gamma and GMCSF, decreased production of IL-10, elevated cellular proliferation and higher differentiation of IFN-gamma-secreting cells. rBCG-mIL-18 also enhanced BCG-induced macrophage cytotoxicity against bladder cancer MBT-2 cells in a dose-dependent manner. Neutralizing all endogenous macrophage-derived cytokines tested (IL-12, IL-18 and TNF-alpha) as well as IFN-gamma severely diminished the rBCG-mIL-18-induced macrophage cytolytic activity, indicating a critical role for these cytokines in this process. Cytokine analysis for supernatants of macrophage-BCG mixture cultures manifested higher levels of IFN-gamma and TNF-alpha in rBCG-mIL-18 cultures than in control BCG cultures. Taken together, this rBCG-mIL-18 strain augments BCG’s immunostimulatory property and may serve as a better agent for bladder cancer immunotherapy and antimycobacterial immunization.—Authors’ Abstract


BACKGROUND: The variable efficacy of bacillus Calmette-Guérin (Mycobacterium bovis BCG) in protecting humans against tuberculosis has prompted a search for the mechanisms through which BCG induces chemokines. In this study, our experiments were designed to determine the role of the transcription factor nuclear factor-kappaB (NF-kappaB) and intracellular calcium in the production of interleukin (IL)-8, a main chemotactic factor, by human-derived monocytic cell line U937 and by a human epithelial HEp-2 cell line infected with M. bovis BCG. METHODS: The concentrations of IL-8 in culture supernatants of U937 cells or HEp-2 cells infected with M. bovis BCG were determined by enzyme-linked immunosorbent assay. We used sulfasalazine and curcumin, which are well-described inhibitors of NF-kappaB activity, and we used ethylenediamine tetraacetic acid to deplete extracellular Ca2+ or used the cell-permeable agent 1,2-bis (2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid tetra (acetoxy-methyl) ester to chelate releasable intracellular stores of Ca2+ in order to investigate the mechanisms through which M. bovis BCG induces IL-8 secretion in our system. RESULTS: The enzyme-linked immunosorbent assay showed that IL-8 protein secretion was elevated in M. bovis-infected cell lines. This effect was statistically significant (p <0.01). When calcium influx was suppressed in M. bovis-infected cell lines, IL-8 secretion was inhibited. Notably, specific inhibitors of NF-kappaB (sulfasalazine and curcumin) inhibited M. bovis-induced IL-8 secretion from U937 cells or HEp-2 cells. CONCLUSIONS: Collectively, these results indicate that activation of NF-kappaB is an important signal transduction pathway in M. bovis-induced IL-8 secretion in monocytic or epithelial cells. Furthermore, the results showed that calcium influx had a direct effect on IL-8 secretion in U937 cells or HEp-2 cells infected with M. bovis.—Authors’ Abstract


The fibronectin-attachment protein (FAP) is conserved among several species of mycobacteria. Although this protein is associated with attachment and internalization of bacteria to host cells via fibronectin, the...
physiological role of the protein still remains unclear. To investigate this point, we generated FAP gene disruptant in \textit{Mycobacterium smegmatis}. The gene disruption, verified by Southern blot and PCR analysis, induced changes on the bacteria, which are associated with strong aggregation and alteration of cell surface properties. Increased hydrophobicity and Congo red accumulation was observed in the FAP gene disruptant. In addition, the complementation experiment demonstrated that the corresponding gene restored wild type morphology in the disruptant. These results indicate that the FAP affects the cell surface properties, and its deletion lead to enhanced aggregation of the \textit{M. smegmatis}.—Authors’ Abstract


The growth of pathogenic mycobacteria in phagosomes of the host cell correlates with their ability to prevent phagosome maturation. The underlying molecular mechanism remains elusive. In a previous study, we have shown that \textit{Mycobacterium avium} depletes the phagosome membrane of cell surface-derived glycoconjugates (de Chastellier and Thilo, Eur. J. Cell Biol. \textit{81}, 17–25, 2002). We now extended these quantitative observations to the major human pathogen, \textit{Mycobacterium tuberculosis} (H37Rv). At increasing times after infection of mouse bone marrow-derived macrophages, cell-surface glycoconjugates were labelled enzymatically with [3H]galactose. Subsequent endocytic membrane traffic resulted in a redistribution of this label from the cell surface to endocytic membranes, including phagosomes. The steady-state distribution was measured by quantitative autoradiography at the electron microscope level. Relative to early endosomes, with which phagosomes continued to fuse and rapidly exchange membrane constituents, the phagosome membrane was depleted about 3-fold, starting during infection and in the course of 9 days thereafter. These results were in quantitative agreement with our previous observations for \textit{Mycobacterium avium}. For the latter case, we now showed by cell fractionation that the depletion was selective, mainly involving glycoproteins in the 110–210 kDa range. Together, these results indicated that pathogenic mycobacteria induced and maintained a bulk change in phagosome membrane composition that could be of special relevance for survival of pathogenic mycobacteria within phagosomes.—Authors’ Abstract


The outcome of Mycobacterium infection is determined by a series of complex interactions between the bacteria and host immunity. Traditionally, mammalian models and cultured cells have been used to study these interactions. Recently, ameba (Dictyostelium), fruit flies (Drosophila) and zebrafish, amenable to forward genetic screens, have been developed as models for mycobacterial pathogenesis. Infection of these hosts with mycobacteria has allowed the dissection of intracellular trafficking pathways (Dictyostelium) and the roles of phagocytic versus antimicrobial peptide responses (Drosophila). Real-time visualization of the optically transparent zebrafish embryo/larva has elucidated mechanisms by which Mycobacterium-infected leukocytes migrate and subsequently aggregate into granulomas, the hallmark pathological structures of tuberculosis.—Authors’ Abstract


The control of \textit{Mycobacterium tuberculosis} infection depends on recognition of the
pathogen and the activation of both the innate and adaptive immune responses. Toll-like receptors (TLR) were shown to play a critical role in the recognition of several pathogens. Mycobacterial antigens recognise distinct TLR resulting in rapid activation of cells of the innate immune system. Recent evidence from in vitro and in vivo investigations, summarized in this review demonstrates TLR-dependent activation of innate immune response, while the induction of adaptive immunity to mycobacteria may be TLR independent.—Authors’ Abstract


The molecular events that occur at the early phase of many demyelinating neurodegenerative diseases are unknown. A recent demonstration of rapid demyelination and axonal injury induced by Mycobacterium leprae provides a model for elucidating the molecular events of early nerve degeneration which might be common to neurodegenerative diseases of both infectious origin and unknown etiology. The identification of the M. leprae-targeted Schwann cell receptor, dystroglycan, and its associated molecules in myelination, demyelination and axonal functions suggests a role for these molecules in early nerve degeneration.—Author’s Abstract


Fifty million new infections with Mycobacterium tuberculosis occur annually, claiming 2–3 million lives from tuberculosis worldwide. Despite the apparent lack of significant genetic heterogeneity between strains of M. tuberculosis, there is mounting evidence that considerable heterogeneity exists in molecules important in disease pathogenesis. These differences may manifest in the ability of some isolates to modify the host cellular immune response, thereby contributing to the observed diversity of clinical outcomes. Here we describe the identification and functional relevance of a highly biologically active lipid species—a polyketide synthase-derived phenolic glycolipid (PGL) produced by a subset of M. tuberculosis isolates belonging to the W-Beijing family that show “hyperlethality” in murine disease models. Disruption of PGL synthesis results in loss of this hypervirulent phenotype without significantly affecting bacterial load during disease. Loss of PGL was found to correlate with an increase in the release of the pro-inflammatory cytokines tumour necrosis factor-alpha and interleukins 6 and 12 in vitro. Furthermore, the overproduction of PGL by M. tuberculosis or the addition of purified PGL to monocyte-derived macrophages was found to inhibit the release of these pro-inflammatory mediators in a dose-dependent manner.—Authors’ Abstract


Current attempts to find a vaccine for tuberculosis (TB) are based on the assumption that it must drive a Th1 response. We review the evidence that progressive disease might not be due to absence of Th1, but rather to the subversive effect of an unusual Th2-like response, involving interleukin-4 (IL-4) and IL-4delta2. This Th2-like response can impair bac tericidal function and lead to toxicity of tumour necrosis factor-alpha (TNF-alpha) and to pulmonary fibrosis. If this is important, effective vaccines will need to suppress pre-existing Th2-like activity. Such vaccines are feasible and are active therapeutically in mouse TB.—Authors’ Abstract

We have previously shown that *Mycobacterium tuberculosis* attenuates cell surface expression of major histocompatibility complex class II molecules in response to gamma interferon (IFN-gamma) by a mechanism dependent on intracellular sequestration of alpha,beta dimers. In this study we examined whether intracellular alkalinization due to mycobacterial urease could account for the defect in intracellular trafficking of class II molecules. Phagocytosis of wild-type *Mycobacterium bovis* BCG was associated with secretion of ammonia intracellularly, which increased substantially upon addition of exogenous urea to the culture medium. Increased intracellular ammonia, due to urea degradation by the bacterium, correlated with inhibition of class II surface expression. Conversely, no ammonia was detected in cells infected with a urease-negative mutant strain of *M. bovis* BCG, which also displayed a reduced effect on surface expression of class II molecules. A direct cause-effect relationship between urease and class II molecule trafficking was established with experiments where cells ingesting beads coated with purified urease showed an increased ammonia level and decreased surface expression of class II in response to IFN-gamma. In contrast to BCG, infection of macrophages with *Mycobacterium smegmatis*, which expresses relatively greater urease activity in cell-free culture, had a marginal effect on both the intracellular level of ammonia and class II expression. The limited effect of *M. smegmatis* was consistent with a failure to resist intracellular killing, suggesting that urease alone is not sufficient to resist macrophage microbicidal mechanisms and that this is required for a more distal effect on cell regulation. Our results demonstrate that alkalinization of critical intracellular organelles by pathogenic mycobacteria expressing urease contributes significantly to the intracellular retention of class II dimers.—Authors’ Abstract


Although post-translational modifications of protein antigens may be important components of some B cell epitopes, the determinants of T cell immunity are generally nonmodified peptides. Here we show that methylation of the *Mycobacterium tuberculosis* heparin-binding hemagglutinin (HBHA) by the bacterium is essential for effective T cell immunity to this antigen in infected healthy humans and in mice. Methylated HBHA provides high levels of protection against *M. tuberculosis* challenge in mice, whereas nonmethylated HBHA does not. Protective immunity induced by methylated HBHA is comparable to that afforded by vaccination with bacille Calmette et Guérin, the only available anti-tuberculosis vaccine. Thus, post-translational modifications of proteins may be crucial for their ability to induce protective T cell-mediated immunity against infectious diseases such as tuberculosis.—Authors’ Abstract


See Current Literature, Chemotherapy, p. 515.

**Immunopathology (Leprosy)**


*Mycobacterium leprae*, an obligate intracellular pathogen, shows a unique tropism for Schwann cells (SC). This leads to the pe-
Peripheral neuropathy disorder observed in leprosy. In this study, we investigated signal transduction events and the intracellular fate of *M. leprae* during the interaction of the microorganism with SC. First, we demonstrated that the human schwannoma cell line ST88-14 readily phagocytized the bacteria as observed by time-lapse microscopy, actin staining and electron microscopy. The effect of specific kinase inhibitors on *M. leprae* internalization was then investigated showing that functional protein tyrosine kinase, calcium-dependent protein kinase and phosphatidylinositol 3-kinase, but not cAMP-dependent protein kinase are essential for phagocytosis of the bacteria. Similar results were obtained when irradiated and live bacteria were compared and when *M. leprae* was pre-coated with recombinant histone-like-protein/ laminin binding protein, a bacterial adhesin. In addition, experiments were performed to analyze the bacterial trafficking within the endosomal network by labeling the acidified intracellular compartments of *M. leprae*-infected SC with the Lyso-tracker acidotrophic probe. Acidification of vesicles containing live *M. leprae* was minimal in both RAW murine macrophages and SC, although phagosomes containing heat-killed bacteria seem to follow normal endocytic maturation. These data indicate that the invading bacteria interfere with normal endocytic pathway maturation of bacteria-containing phagosomes within SC.—Authors’ Abstract


The lepromatous leprosy granuloma is a dynamic entity requiring a steady influx of macrophages (Mphi) for its maintenance. We have developed an in vitro model to study the fate of *Mycobacterium leprae* in a LL lesion, with and without immunotherapeutic intervention. Target cells, consisting of granuloma Mphi harvested from the footpads of *M. leprae*-infected athymic nu/nu mice, were cocultured with normal or IFN-gamma-activated (ACT) effector Mphi. The bacilli were recovered and assessed for viability by radiorepirometry. *M. leprae* recovered from target Mphi possessed high metabolic activity, indicating a viable state in this uncultivable organism. *M. leprae* recovered from target Mphi incubated with normal effector Mphi exhibited significantly higher metabolism. In contrast, bacilli recovered from target Mphi cocultured with ACT effector Mphi displayed a markedly decreased metabolic activity. Inhibition by ACT Mphi required an E:T ratio of at least 5:1, a coculture incubation period of 3–5 days, and the production of reactive nitrogen intermediates, but not reactive oxygen intermediates. Neither IFN-gamma nor TNF-alpha were required during the cocultivation period. However, cell-to-cell contact between the target and effector Mphi was necessary for augmentation of *M. leprae* metabolism by normal effector Mphi as well as for inhibition of *M. leprae* by ACT effector Mphi. Conventional fluorescence microscopy and confocal fluorescence microscopy revealed that the bacilli from the target Mphi were acquired by the effector Mphi. Thus, the state of Mphi infiltrating the granuloma may markedly affect the viability of *M. leprae* residing in Mphi in the lepromatous lesion.—Authors’ Abstract


Toll-like receptor 2 (TLR2) is a key mediator of the immune response to mycobacterial infections, and mutations in TLR2 have been shown to confer susceptibility to infection with mycobacteria. This study investigated the profiles of cytokines, such as interferon (IFN)-gamma, interleukin (IL)-10, IL-12 and tumour necrosis factor (TNF)-alpha in response to *Mycobacterium leprae* in peripheral blood mononuclear cells (PBMC) with the TLR2 mutation Arg677Trp, a recently reported polymorphism that is associated with lepromatous leprosy. In leprosy patients with the TLR2 mutation, production of IL-2, IL-12, IFN-
gamma, and TNF-alpha by *M. leprae*-stimulated PBMC were significantly decreased compared with that in groups with wild-type TLR2. However, the cells from patients with the TLR2 mutation showed significantly increased production of IL-10. There was no significant difference in IL-4 production between the mutant and wild-type during stimulation. Thus, these results suggest that the TLR2 signal pathway plays a critical role in the alteration of cytokine profiles in PBMC from leprosy patients and the TLR2 mutation Arg677Trp provides a mechanism for the poor cellular immune response associated with lepromatous leprosy.—Authors’ Abstract


RIPK 2 is adapter molecule in the signal pathway involved in Toll-like receptors. However, there has been no reported association between receptor-interacting serine/threonine kinase 2 (RIPK 2) expression and the infectious diseases involving mycobacterial infection. This study found that its expression was down-regulated in the footpads and skin but was up-regulated in the liver of *Mycobacterium leprae*-infected nu/nu mice compared with those of the *M. leprae* non-infected nu/nu mice. It was observed that the interleukin-12p40 and interferon-gamma genes involved in the susceptibility of *M. leprae* were down-regulated in the skin but were up-regulated in the liver. Overall, this suggests that regulation of RIPK 2 expression is tissue-specifically associated with *M. leprae* infection.—Authors’ Abstract


Macrophages are one of the most abundant host cells to come in contact with mycobacteria. However, the infected macrophages less efficiently stimulate autologous T cells *in vitro*. We investigated the effect of the induction of phenotypic change of macrophages on the host cell activities by using *Mycobacterium leprae* as a pathogen. The treatment of macrophages with interferon-gamma (IFN-gamma), GM-CSF and interleukin-4 deprived macrophages of CD14 antigen expression but instead provided them with CD1a, CD83 and enhanced CD86 antigen expression. These phenotypic features resembled those of monocyte-derived dendritic cells (DC). These macrophage-derived DC-like cells (MACDC) stimulated autologous CD4+ and CD8+ T cells when infected with *M. leprae*. Further enhancement of the antigen-presenting function and CD1a expression of macrophages was observed when treated with IFN-gamma. *The M. leprae*-infected and -treated macrophages expressed bacterial cell membrane-derived antigens on the surface and were efficiently cytolyzed by the cell membrane antigen-specific CD8+ cytotoxic T lymphocytes (CTL). These results suggest that the induction of phenotypic changes in macrophages can lead to the upregulation of host defence activity against *M. leprae*.—Authors’ Abstract


We have determined IL-10 promoter genotypes of five single-nucleotide polymorphisms (SNPs): T-3575A, A-2849G, C-2763A, -A-1082G and C-819T. The haplotype frequencies were defined in healthy subjects compared to leprosy patients, and analyzed for their occurrence in multi-(MB) vs paucibacillary (PB) as severe and mild forms of leprosy, respectively. Haplotypes defined by three SNP positions (−3575, −2849 and −2763) captured significant differences between controls and patients (p =
The haplotype carrying –3575A, –2849G and –2763C was associated with resistance to leprosy and to the development of severe forms of the disease using either a binomial (controls vs. cases, p = 0.005, OR = 0.35, CI = 0.13–0.91) or ordinal (controls vs PB vs MB, p = 0.006, OR = 0.32, CI = 0.12–0.83) model. By contrast, the IL-10 haplotype –3575T/–2849A/–2763C was found to be associated with susceptibility to leprosy per se (p = 0.027, OR = 2.37, CI = 1.04–5.39), but not leprosy type. The data suggest that the IL-10 locus contributes to the outcome of leprosy.—Authors’ Abstract


Leprosy is characterized by a wide spectrum of clinical features depending on the individual differences in Th1-type immunity. The objective of this study was to evaluate whether monocyte activation by stimulus via class II HLA molecules would be correlated with the differences in cellular immune responses among diverse clinical forms of leprosy. IL-1beta and IL-12 productivity in monocyte preparations obtained from PBMCs was estimated in patients with lepromatous- and tuberculoid-type leprosy. We found that monocytes from lepromatous patients produced significantly higher (about 4-fold higher) amounts of IL-12 as compared to in patients with tuberculoid type of leprosy when class II HLA molecules were cross-linked with anti-HLA class II antibodies, whereas almost equal amounts of IL-1beta were produced from each monocyte preparation by stimulus via class II HLA molecules regardless of the clinical form of leprosy. These results suggest that monocyte activation differs between lepromatous and tuberculoid patients in terms of IL-12 secretion, which might be related to individual differences in the cellular immune responses according to the clinical type of leprosy.—Authors’ Abstract


Severe oxidative stress has been reported in leprosy patients because of malnutrition and poor immunity. The purpose of this study was to investigate the serum lipid peroxidation products, serum LDH and important free radical scavenging enzymes, i.e. superoxide dismutase (SOD), and catalase and anti-oxidant glutathione levels and total anti-oxidant status, in different types of leprosy patients. The subjects for this study were normal human volunteers (NHVs, N = 14), paucibacillary leprosy patients (PB, N = 18), untreated MB patients (MB1, N = 18), MB patients under treatment (MB2, N = 19), and MB patients released from treatment (RFT) (MB3, N = 28). The levels of lipid peroxidation product, malondialdehyde (MDA), and LDH increased significantly (p <0.001) in MB (MB1, MB2, MB3) patients, in comparison with NHVs. They gradually increased with clinical improvement with MDT. There was no significant variation of these parameters in PB leprosy patients in comparison with healthy volunteers. High free radical activity and low anti-oxidant levels observed in MB (MB1, MB2, MB3) leprosy patients indicate that there is an oxidative stress in MB cases, irrespective of the treatment status and suggest a suitable anti-oxidant therapy to prevent possible tissue injury.—Authors’ Abstract


Some mycobacterial infections, such as tuberculosis, are characterized by apoptosis of infected or by-stander mononuclear immune cells. For localized (paucibacillary,
PB) and disseminated (multibacillary, MB) leprosy, characterized by polarized Th1-like vs. Th2-like immune responses, respectively, little is known about lesional apoptosis. We analyzed sections of paraffin-embedded, untreated leprosy lesions from 21 patients by an indirect immunofluorescent terminal deoxynucleotide-transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) assay. Some TUNEL (+) PB sections were then reacted with phycoerythrin-conjugated (red) antibodies against T cells, monocytes, or antigen-presenting (Langerhans) cells. TUNEL (+) bodies were detected in 9 of 16 PB lesions (56%) and in 1 of 5 MB lesions (20%). Some TUNEL (+) bodies in PB disease were CD3+ (T cell), as well as CD4+ (T-helper) or CD8+ (T-cytotoxic). Apoptosis characterizes PB and MB leprosy lesions and may be more frequent in PB disease. In PB disease, some TUNEL (+) bodies may derive from T cells.—Authors’ Abstract


*Mycobacterium leprae* lipoprotein, LpK, induced IL-12 production from human monocytes. To determine the components essential for cytokine production and the relative role of lipidation in the activation process, we produced lipidated and non-lipidated truncated forms of LpK. While 0.5 nM of lipidated LpK-a having N-terminal 60 amino acids of LpK produced more than 700 pg/ml IL-12 p40, the non-lipidated LpK-b having the same amino acids as that of LpK-a required more than 20nM of the protein to produce an equivalent dose of cytokine. Truncated protein having the C-terminal 192 amino acids of LpK did not induce any cytokine production. Fifty nanomolar of the synthetic lipopeptide of LpK produced only about 200pg/ml IL-12. Among the truncated LpK, only LpK-a and lipopeptide stimulated NF-kB-dependent reporter activity in TLR-2 transfectant. However, when monocytes were stimulated with lipopeptide in the presence of non-lipidated protein, they produced IL-12 synergistically. Therefore, both peptide regions of LpK and lipid residues are necessary for efficient IL-12 production.—Authors’ Abstract

Immunopathology (Tuberculosis)


Proteins released from *Mycobacterium tuberculosis* (Mtb) during late logarithmic growth phase are often considered candidate components of immunogenic or autolysis markers. One such protein is isocitrate dehydrogenase (ICD), a key regulatory enzyme in the citric acid cycle. We have evaluated the immunogenic properties of two isoforms of Mtb ICD and compared them with the control antigens heat-shock protein 60 and purified protein derivative (PPD). PPD lacks the sensitivity to distinguish between bacillus Calmette-Guérin (BCG)-vaccinated and tuberculosis (TB)-infected populations, and, therefore, epidemiological relevance of PPD in BCG-vaccinated regions is debatable. We show that Mtb ICDs elicit a strong B cell response in TB-infected populations and can differentiate between healthy BCG-vaccinated populations and those with TB. The study population (N = 215) was categorized into different groups, namely, patients with fresh infection (N = 42), relapsed TB cases (N = 32), patients with extrapulmonary TB (N = 35), clinically healthy donors (N = 44), nontuberculous mycobacteria patients (N = 30), and non-TB
patients (culture negative for acid-fast bacteria but carrying other infections, N = 32). The Mtb ICDs showed statistically significant antigenic distinction between healthy BCG-vaccinated controls and TB patients (p <0.0001) and those with other infections. Although extrapulmonary infections could not be discriminated from healthy controls by heat-shock protein 60 (p = 0.2177), interestingly, the Mtb ICDs could significantly (p <0.0001) do so. Our results highlight the immunodominant, immunosensitive, and immunospecific nature of Mtb ICDs and point to an unusual property of this tricarboxylic acid energy cycle enzyme.—Authors’ Abstract


The differential transcriptional response of Mycobacterium tuberculosis to drugs and growth-inhibitory conditions was monitored to generate a data set of 430 microarray profiles. Unbiased grouping of these profiles independently clustered agents of known mechanism of action accurately and was successful at predicting the mechanism of action of several unknown agents. These predictions were validated biochemically for two agents of previously uncategorized mechanism, pyridoacridones and phenothiazines. Analysis of this data set further revealed 150 underlying clusters of coordinately regulated genes offering the first glimpse at the full metabolic potential of this organism. A signature subset of these gene clusters was sufficient to classify all known agents as to mechanism of action. Transcriptional profiling of both crude and purified natural products can provide critical information on both mechanism and detoxification prior to purification that can be used to guide the drug discovery process. Thus, the transcriptional profile generated by a crude marine natural product recapitulated the mechanistic prediction from the pure active component. The underlying gene clusters further provide fundamental insights into the metabolic response of bacteria to drug-induced stress and provide a rational basis for the selection of critical metabolic targets for screening for new agents with improved activity against this important human pathogen.—Authors’ Abstract


The cell wall component lipoarabinomannan (ManLAM) from Mycobacterium tuberculosis is involved in the inhibition of phagosome maturation, apoptosis and interferon (IFN)-gamma signalling in macrophages and interleukin (IL)-12 cytokine secretion of dendritic cells (DC). All these processes are important for the host to mount an efficient immune response. Conversely, LAM isolated from non-pathogenic mycobacteria (PILAM) have the opposite effect, by inducing a potent proinflammatory response in macrophages and DCs. LAMs from diverse mycobacterial species differ in the modification of their terminal arabinose. The strong proinflammatory response induced by PILAM correlates with the presence of phospho-myo-inositol on the terminal arabinose. Interestingly, recent work indicates that the biosynthetic precursor of LAM, lipomannan (LM), which is also present in the cell wall, displays strong proinflammatory effects, independently of which mycobacterial species it is isolated from. Results from in vitro assays and knock-out mice suggest that LM, like PILAM, mediates its biological activity via Toll-like receptor 2. We hypothesize that the LAM/LM ratio might be a crucial factor in determining the virulence of a mycobacterial species and the outcome of the infection. Recent progress in the identification of genes involved in the biosynthesis of LAM is discussed, in particular with respect to the fact that enzymes controlling the LAM/LM balance might represent targets for new antitubercular drugs. In addition, inactivation of these genes may lead to attenuated strains of
M. tuberculosis for the development of new vaccine candidates.—Authors’ Abstract


The potent human pathogen Mycobacterium tuberculosis persists in macrophages within a specialized, immature phagosome by interfering with the pathway of phagolysosome biogenesis. The molecular mechanisms underlying this process remain to be fully elucidated. Here, using four-dimensional microscopy, we detected on model phagosomes, which normally mature into phagolysosomes, the existence of cyclical waves of phosphatidylinositol 3-phosphate (PI3P), a membrane trafficking regulatory lipid essential for phagosomal acquisition of lysosomal characteristics. We show that mycobacteria interfere with the dynamics of PI3P on phagosomal organelles by altering the timing and characteristics of the PI3P waves on phagosomes. The default program of cyclical PI3P waves on model phagosomes is composed of an initial stage (phase I), represented by a strong PI3P burst occurring only upon the completion of phagosome formation, and a subsequent stage (phase II) of recurring PI3P waves on maturing phagosomes with the average periodicity of 20 min. Mycobacteria alter this program in two ways: (i) by inducing, in a cholesterol-dependent fashion, a neophase I* of premature PI3P production, coinciding with the process of mycobacterial entry into the macrophage, and (ii) by inhibiting the calmodulin-dependent phase II responsible for the acquisition of lysosomal characteristics. We conclude that the default pathway of phagosomal maturation into the phagolysosome includes temporally organized cyclical waves of PI3P on phagosomal membranes and that this process is targeted for reprogramming by mycobacteria as they prevent phagolysosome formation.—Authors’ Abstract


Mycobacterium tuberculosis induces apoptosis in human monocyte-derived macrophages (MDMs) during the early stages of infection. We investigated the proapoptotic role of cell wall-associated mycobacterial 19-kDa lipoprotein and the possible association between 19-kDa lipoprotein signaling and production of proinflammatory cytokines. Purified mycobacterial 19-kDa lipoprotein, 19-kDa lipoprotein-expressing M. smegmatis (M. smegmatis 19+), 19-kDa lipoprotein knockout (KO) M. tuberculosis, and 19-kDa lipoprotein KO M. bovis bacille Calmette-Guerin (BCG) strains were analyzed for their ability to induce apoptosis in MDMs. The 19-kDa lipoprotein and infection with M. smegmatis 19+ induced apoptosis in MDMs. M. tuberculosis and BCG KO strains had significantly decreased abilities to induce apoptosis. The 19-kDa lipoprotein proapoptotic signal was mediated by Toll-like receptor 2 but not by tumor necrosis factor-alpha. Only the release of interleukin (IL)-1 beta was decreased after infection with 19-kDa lipoprotein KO strains. These findings indicate that the 19-kDa lipoprotein is the main signal required to trigger both apoptosis and the release of IL-1 beta during the early stages of mycobacterial infection.—Authors’ Abstract


T cell activation in response to antigenic stimulation is a complex process, involving changes in the expression level of a large number of genes. We have used cDNA array
technology to characterize the differences in gene expression between human CD4+ and CD8+ T cells. PBMC from six healthy donors were stimulated with live *Mycobacterium tuberculosis*, and the gene expression profiles of each donor’s CD4+ and CD8+ T cells were analyzed separately. ANOVA revealed 518 genes that were consistently differentially expressed between CD4+ and CD8+ T cells. These differentially expressed genes include a combination of well-known, previously characterized genes with a range of biological functions and unknown in silico predicted hypothetical genes. Where possible, the novel genes have been characterized using bioinformatics, and putative transcription factors, signaling molecules, transmembrane, and secreted factors have been identified. A subset of these differentially expressed genes could be exploited as markers of CD4+ and CD8+ T cell activation for use in vaccine trials. These observed differences in the gene expression profile of CD4+ and CD8+ T cells following activation by a human pathogen contribute to an increased understanding of T cell activation and differentiation and the roles these T cell subsets may play in immunity to infection.—Authors’ Abstract


MHC class II (MHC-II)-restricted CD4(+) T cells are essential for control of *Mycobacterium tuberculosis* infection. This report describes the identification and purification of LprG (Rv1411c) as an inhibitor of primary human macrophage MHC-II Aβ processing. LprG is a 24-kDa lipoprotein found in the *M. tuberculosis* cell wall. Prolonged exposure (>16 hr) of human macrophages to LprG resulted in marked inhibition of MHC-II Ag processing. Inhibition of MHC-II Ag processing was dependent on TLR-2. Short-term exposure (<6 hr) to LprG stimulated TLR-2-dependent TNF-alpha production. Thus, LprG can exploit TLR-2 signaling to inhibit MHC-II Ag processing in human macrophages. Inhibition of MHC-II Ag processing by mycobacterial lipoproteins may allow *M. tuberculosis*, within infected macrophages, to avoid recognition by CD4(+) T cells.—Authors’ Abstract


Arabinomannan (AM) is a polysaccharide of the mycobacterial capsule. The capsular polysaccharides of various microorganisms are diverse, and this diversity is important for classification of organisms into serotypes and vaccine development. In the present study we examined the prevalence and diversity of AM among *Mycobacterium tuberculosis* strains using four AM-binding monoclonal antibodies (MAbs). One of these MAbs, MAb 9d8, is known to bind to AM specifically. By whole-cell enzyme-linked immunosorbent assay (ELISA), the AM recognized by MAb 9d8 was detected on the surfaces of 9 of 11 strains, while 2 strains showed no reactivity with MAb 9d8. However, the AM recognized by MAb 9d8 was found in the culture supernatants of all 11 *M. tuberculosis* strains tested, as demonstrated by capture ELISA. Other AM-binding MAbs reacted both with the surfaces and with the culture supernatants of all 11 strains. Mice immunized with an experimental AM-rEPA vaccine had an increased antibody response to AM and a moderate reduction in the numbers of CFU in their organs 7 days after challenge. Our results indicate that AM was detected in all *M. tuberculosis* strains tested, with differences in epitope distributions of certain strains. In addition, our results suggest that an experimental AM-rEPA vaccine has a moderate effect on the numbers of CFU in organs early after infection.—Authors’ Abstract

The present study defines pathologic differences in acute and hypersensitive responses to Mycobacterium tuberculosis glycolipid trehalose-6,6′-dimycolate (TDM, cord factor) in normal BALB/c mice and those deficient in group II CD1 molecule CD1d1. Mice immunized against TDM demonstrate hypersensitive responses, yet the mechanisms for TDM presentation remain elusive. Mice lacking CD1d (CD1D(−/−)) demonstrate dysregulated granulomatous response to TDM, compared with CD1D(+/−) heterozygous controls. Because CD1d-restricted T cells can regulate macrophage immune functions at mucosal surfaces, we hypothesized that CD1D(−/−) mice would show deficient TDM-induced hypersensitive pulmonary granulomatous response in which T cells play a central role. Control CD1D(++) mice sensitized and subsequently challenged with TDM demonstrated aggressive inflammation defined by monocytic lesions contained by CD3(+) lymphocytic cuffing. CD1D(−/−) mice demonstrated distinctly different pathologies, with edema present concurrent with extended, nonfocal mononuclear cell-based granulomatous reactions. Furthermore, CD1D(−/−) mice did not demonstrate destructive lesions, and CD3(+) lymphocytes were only loosely organized in proximity to reactive pathology. The CD1d-deficient mice demonstrated rapid increases in proinflammatory mRNAs, with significant differences in interferon-gamma (IFN-gamma) compared to the wild-type group. IFN-gamma, interleukin-6 (IL-6), and IL-12 proteins were significantly elevated in the CD1D(−/−) group compared with wild-type mice (p <0.05) 2 days after TDM challenge. However, by 7 days postadministration, similar production for all cytokines and proinflammatory molecules examined was present in both groups of mice. These experiments provide evidence for a role for CD1d in mediation of pathology during hypersensitive responses to the mycobacterial glycolipid TDM.—Authors’ Abstract


In the Mycobacterium tuberculosis H37Rv genome, there are 11 paired two-component regulatory system genes, two orphan histidine kinase genes, and six orphan response regulator genes. Expression of the 17 response regulator genes and the two orphan histidine kinase genes during growth of M. tuberculosis in human peripheral blood monocyte-derived macrophages has been analyzed by using cDNA mixtures prepared by the selective capture of transcribed sequences (SCOTS) technique. SCOTS probes were prepared from cDNA obtained from M. tuberculosis grown for 18, 48, and 110 hr in human macrophages. Based on expression profiles, the regulatory genes were assigned to three categories: (i) constitutively expressed during growth in macrophages (three genes); (ii) differentially expressed during growth in macrophages (nine genes) and (iii) no detectable expression during growth in macrophages (seven genes).—Authors’ Abstract


BACKGROUND: Infection of alveolar macrophages (AMs), which constitute the first line of defense against Mycobacterium tuberculosis, initiates an intense interaction between the host’s innate immune response and mycobacteria that may assist in the successful intracellular parasitism of M. tuberculosis. METHODS: Expression of tumor necrosis factor (TNF)-alpha and M. tuberculosis 85B mRNA was studied in M. tuberculosis-infected AMs, to better delineate the role of macrophages in the early events in initiation of infection. RESULTS:
Both TNF-alpha mRNA and *M. tuberculosis* 85B were induced in AMs; at 24 hr, the time point of maximum TNF-alpha induction, the mRNA levels for TNF-alpha and *M. tuberculosis* 85B correlated with one another, and induction of either gene correlated strongly with their protein levels. Inhibition of endogenous TNF-alpha by soluble (s) TNF receptor (R) I and sTNFRII reduced expression of both TNF-alpha and *M. tuberculosis* 85B. The activation of nuclear factor-kappa B was found to underlie expression of both TNF-alpha and *M. tuberculosis* 85B. Interestingly, inhibition of bactericidal mediators, reactive oxygen intermediates (ROIs) and reactive nitrogen intermediates (RNIs), reduced expression of TNF-alpha and *M. tuberculosis* 85B genes in *M. tuberculosis*-infected AMs. CONCLUSION: Activation of AMs by *M. tuberculosis* initiates a cascade of events whereby TNF-alpha, ROI, and RNI enhance the expression of the *M. tuberculosis* 85B gene.—Authors’ Abstract


Gene expression patterns associated with resistance and susceptibility to tuberculosis (TB) were investigated at the macrophage level in the well-defined mouse model of infection. Oligonucleotide microarrays were used to analyze the regulation of gene expression in murine bone marrow-derived macrophages infected with *Mycobacterium tuberculosis*. Four mouse strains, known to differ in terms of growth permissiveness for *M. tuberculosis* in infected tissues, in the development of pulmonary pathology, and in the rate of premature death due to tuberculosis, were compared: C57BL/6 and BALB/c representing resistant, DBA/2 and CBA/J representing susceptible mouse strains. Genes (55) were regulated more than two-fold in macrophages of all strains investigated following *M. tuberculosis* infection. Importantly, 18 genes were commonly regulated only in macrophages of the two resistant strains upon infection, and 102 genes were commonly regulated exclusively in macrophages of the two susceptible strains. Using this approach, we have identified more than 100 genes potentially associated with resistance and susceptibility, respectively, to TB at the macrophage level. A tentative interpretation of our microarray data suggests that macrophages from susceptible mice predominantly stimulate the recruitment of cells that contribute disproportionately to tissue damage rather than to microbial elimination. In conclusion, microarray gene chips are use-
ful tools for generating new hypotheses about resistance and susceptibility to TB, and the mouse model can now be used to subject candidate genes identified by this approach to further functional analyses.—Authors’ Abstract


In vitro infection of monocytes with Mycobacterium tuberculosis HN878 and related W/Beijing isolates preferentially induced interleukin-4 (IL-4) and IL-13, which characterize Th2 polarized immunity. In contrast, CDC1551 induced more IL-12 and other molecules associated with phagocyte activation and Th1 protective immunity. The differential cytokine-chemokine response was mediated by extracted lipids, suggesting that these molecules regulate host responses to infection.—Authors’ Abstract


Mycobacterium tuberculosis (Mtb) is an extraordinarily successful human pathogen, one of the major causes of death by infectious disease worldwide. A key issue for the study of tuberculosis is to understand why individuals infected with Mtb experience different clinical outcomes. To better understand the dynamics of Mtb infection and immunity, we coupled nonhuman primate experiments with a mathematical model we previously developed that qualitatively and quantitatively captures important processes of cellular priming and activation. These processes occur between the lung and the nearest draining lymph node where the key cells mediating this process are the dendritic cells (DC). The nonhuman primate experiments consist of bacteria and cell numbers from tissues of 17 adult cynomolgus macaques (Macaca fascicularis) that were infected with Mt strain Erdman (approximately 25 CFU/animal via bronchoscope). The main result of this work is that delays in either DC migration to the draining lymph node or T cell trafficking to the site of infection can alter the outcome of Mtb infection, defining progression to primary disease or latent infection and reactivated tuberculosis. Our results also support the idea that the development of a new generation of treatment against Mtb should optimally elicit a fast DC turnover at the site of infection, as well as strong activation of DCs for maximal Ag presentation and production of key cytokines. This will induce the most protective T cell response.—Authors’ Abstract


We addressed the question of whether protective immunity induced by natural infection with Mycobacterium tuberculosis and that induced by vaccination with Mycobacterium bovis bacille Calmette-Guerin (BCG) differ in the murine model. We infected mice with M. tuberculosis Erdman, cured them by chemotherapy, and subsequently reinfected them with a low dose of M. tuberculosis H37Rv. The course of tuberculosis was compared with that in mice previously vaccinated with BCG Danish 1331. Protection against postprimary M. tuberculosis infection did not differ significantly between the 2 groups. After challenge infection, numbers of interferon- gamma-positive splenocytes did not differ between mice with primary infection and vaccinated mice. Splenocytes from primary M. tuberculosis-infected mice conferred marginally higher protection than did those from BCG-vaccinated mice. Serum transfer did not protect against reinfection in either group. Our data emphasize that natural infection with M. tuberculosis and vaccination with BCG do not differ in their capacity to induce pro-
tective immunity against tuberculosis and support the notions that reinfection contributes to the development of active disease and that any novel vaccine against tuberculosis has to perform better than both vaccination with BCG and immunity evoked by natural infection.—Authors’ Abstract


We previously reported that CCR2(–/–) mice are susceptible to Mycobacterium tuberculosis infection. Susceptibility was associated with an early and sustained macrophage trafficking defect, followed by delayed recruitment of dendritic cells (DCs) and T cells to the lungs. However, the relative importance of the lack of CCR2 expression by macrophages and DCs vs T cells in susceptibility to infection was unclear. In this study, we used mixed bone marrow transplantation to create mice in which the genotype of the T cells was either CCR2(+/+) or CCR2(–/–) while maintaining the genotype of the myeloid cells as CCR2(+/+). After infection with M. tuberculosis, we found that the genotype of the macrophages and/or DCs was critical for both T cell and myeloid cell migration to the lungs. Further investigation revealed a critical role for CCR2 in the recruitment of F4/80(dim) macrophages and CD11c (dim/intermediate) DCs to the infected lung.—Authors’ Abstract


Lipoproteins are a subgroup of secreted bacterial proteins characterized by a lipidated N-terminus, processing of which is mediated by the consecutive activity of pro-lipoprotein diacylglycerol transferase (Lgt) and lipoprotein signal peptidase (LspA). The study of LspA function has been limited mainly to non-pathogenic microorganisms. To study a potential role for LspA in the pathogenesis of bacterial infections, we have disrupted LspA by allelic replacement in Mycobacterium tuberculosis, one of the world’s most devastating pathogens. Despite the presence of an impermeable lipid outer layer, it was found that LspA was dispensable for growth under in vitro culture conditions. In contrast, the mutant was markedly attenuated in virulence models of tuberculosis. Our findings establish lipoprotein metabolism as a major virulence determinant of tuberculosis and define a role for lipoprotein processing in bacterial pathogenesis. In addition, these results hint at a promising new target for therapeutic intervention, as a highly specific inhibitor of bacterial lipoprotein signal peptidases is available.—Authors’ Abstract


Tumour necrosis factor (TNF) is critical for sustained protective immunity against Mycobacterium tuberculosis infection. To investigate the relative contributions of macrophage- and T cell-derived TNF towards this immunity T cells from wild-type (WT) or TNF–/– mice were transferred into RAG–/– or TNF–/– mice which were then infected with M. tuberculosis. Infected RAG–/– mice and RAG–/– recipients of TNF deficient T cells developed overwhelming infection, with extensive pulmonary and hepatic necrosis and succumbed with a median of only 16 days infection. By contrast, RAG–/– recipients of WT T cells showed a significant increase in survival with a median of 32 days. Although initial bacterial growth was similar in all groups of RAG–/– mice, the transfer of WT, but not TNF–/–, T cells led to the formation of discrete foci of leucocytes and macrophages.
and delayed the development of necrotizing pathology. To determine requirements for macrophage-derived TNF, WT or TNF−/− T cells were transferred into TNF−/− mice at the time of *M. tuberculosis* infection. Transfer of WT T cells significantly prolonged survival and reduced the early tissue necrosis evident in the TNF−/− mice, however, these mice eventually succumbed indicating that T cell-derived TNF alone is insufficient to control the infection. Therefore, both T cell- and macrophage-derived TNF play distinct roles in orchestrating the protective inflammatory response and enhancing survival during *M. tuberculosis* infection.
—Authors’ Abstract


The host effector mechanisms against *Mycobacterium tuberculosis* infection are not well understood, and this remains a problem in the development of new vaccines and immunotherapies in tuberculosis (TB). Here, we studied the expression of genes for interferon-gamma (IFN-gamma) and molecules involved in lymphocyte-mediated cytotoxicity by *Mycobacterium tuberculosis* and during human tuberculosis. Scand. J. Immunol. 60(3) (2004) 299–306.

The host effector mechanisms against *Mycobacterium tuberculosis* infection are not well understood, and this remains a problem in the development of new vaccines and immunotherapies in tuberculosis (TB). Here, we studied the expression of genes for interferon-gamma (IFN-gamma) and molecules involved in lymphocyte-mediated cytotoxicity (granzyme B (grzB), perforin, granulysin and Fas ligand (FasL)) against *M. tuberculosis*-infected macrophages. The kinetics of expression of these molecules were first established in peripheral blood mononuclear cells (PBMC) of healthy donors, and then investigated in TB patients with and without HIV-1 coinfection and appropriate control groups. We found that only IFN-gamma and grzB were induced by *M. tuberculosis* in PBMC from healthy purified protein derivative skin test reactive subjects. However, expression of neither gene nor IFN-gamma protein correlated with intracellular *M. tuberculosis* growth containment by macrophages. *Mycobacterium tuberculosis* induction of IFN-gamma, but not grzB, mRNA expression was significantly lower (p <0.03) in TB patients as compared with healthy subjects. —Authors’ Abstract


Phagocytosis and phagolysosome biogenesis represent fundamental biological processes essential for proper tissue homeostasis, development, elimination of invading microorganisms, and antigen processing and presentation. Phagosome formation triggers a pre-programmed pathway of maturation into the phagolysosome, a process controlled by Ca2+ and the regulators of organelar trafficking centered around the small GTP-binding proteins Rabs and their downstream effectors, including lipid kinases, organelar tethering molecules, and membrane fusion apparatus. *Mycobacterium tuberculosis* is a potent human pathogen parasitizing macrophages. It interferes with the Rab-controlled membrane trafficking and arrests the maturing phagosome at a stage where no harm can be done to the pathogen while the delivery of nutrients and membrane to the vacuole continues harboring the microorganism. This process, referred to as the *M. tuberculosis* phagosome maturation arrest or inhibition of phagosome-lysosome fusion, is critical for *M. tuberculosis* persistence in human populations. It also provides a general model system for dissecting the phagolysosome biogenesis pathways. Here we review the fundamental trafficking processes targeted by *M. tuberculosis* and the mycobacterial products that interfere with phagosomal maturation. Expected online publication date for the Annual Review of Cell and Developmental Biology Volume 20 is October 6, 2004.—Authors’ Abstract


We have developed a colorimetric microtitre plate hybridization assay in order to simplify detection of *Mycobacterium leprae* in clinical specimens. This system detects the products amplified by a sensitive RT-
PCR assay targeting a species-specific sequence of the bacterial 16S rRNA. The assay detected as few as 10 bacilli isolated from infected nude mouse lymph nodes or human skin biopsies. Sensitivity for diagnosis of clinical specimens was assessed for 58 tissue biopsies from untreated leprosy patients seen at the ALERT Hospital in Addis Ababa, Ethiopia [date not given]. The assay detected \( M. \text{leprae} \) RT-PCR products in 100% of biopsies from patients with multibacillary disease and 80% of biopsies from patients with paucibacillary disease, for an overall sensitivity of 91.3%. The test was highly specific as no RT-PCR products were amplified from skin biopsies of normal individuals or patients with skin diseases other than leprosy. The colorimetric assay is faster, more sensitive, and simplifies detection of RT-PCR products compared to Southern blot analysis. It may be useful for diagnosis of difficult cases of leprosy, and, since RNA is rapidly degraded after cell death, it may be appropriate for assessing response to therapy and for distinguishing relapse from reaction.—Tropical Disease Bulletin

**Microbiology**


Conjugal DNA transfer occurs by an atypical mechanism in \( \text{Mycobacterium smegmatis} \). The transfer system is chromosomally encoded and requires recipient recombination functions for both chromosome and plasmid transfer. Cis-acting sequences have been identified that confer mobility on non-transferable plasmids, but these are larger and have different properties to canonical oriT sites found in bacterial plasmids. To identify trans-acting factors required for mediating DNA transfer, a library of transposon insertion mutants was generated in the donor strain, and individual mutants were screened for their effect on transfer. From this screen, a collection of insertion mutants was isolated that increased conjugation frequencies relative to wild type. Remarkably, the mutations map to a 25-kb region of the \( M. \text{smegmatis} \) chromosome that is syntenous with the RD1 region of \( \text{Mycobacterium tuberculosis} \), which is considered to be the primary attenuating deletion in the related vaccine strain \( \text{Mycobacterium bovis} \) bacillus Calmette-Guérin. The genes of the RD1 region encode a secretory apparatus responsible for exporting Cfp10- and Esat-6, both potent antigens and virulence factors. In crosses using two \( \text{M. smegmatis} \) donors, we show that wild-type cells can suppress the elevated transfer phenotype of mutant donors, which is consistent with the secretion of a factor that suppresses conjugation. Most importantly, the RD1 region of \( M. \text{tuberculosis} \) complements the conjugation phenotype of the RD1 mutants in \( M. \text{smegmatis} \). Our results indicate that the \( M. \text{tuberculosis} \) and \( M. \text{smegmatis} \) RD1 regions are functionally equivalent and provide a unique perspective on the role of this critical secretion apparatus.—Authors’ Abstract

**Lee, R. E., Li, W., Chatterjee, D., and Lee, R. E.** Rapid structural characterization of the arabinogalactan and lipoarabinomannan in live mycobacterial cells using 2D and 3D HR-MAS NMR. Glycobiology. Epub 2004 Sep. 15 [ahead of print]

Mycobacteria possess a unique, highly evolved, carbohydrate and lipid-rich cell wall that is believed to be important for their survival in hostile environments. Until now, our understanding of mycobacterial cell wall structure has been based upon destructive isolation and fragmentation of individual cell wall components. This study describes the observation of the major cell wall structures in live, intact mycobacteria using two-dimensional (2D) and three-dimensional (3D) High-Resolution Magic-Angle Spinning (HR-MAS) NMR. As little as 20 mg (wet weight) of [(13)C] enriched
cells were required to produce a whole-cell spectra in which discrete cross-peaks corresponding to specific cell wall components could be identified. The most abundant signals of the arabinoxylan (AG) and lipopolysaccharide (LPS) were assigned in the HR-MAS NMR spectra by comparing the 2D and 3D NMR whole-cell spectra with the spectra of purified cellular components. This study confirmed that the structures of the AG and LAM moieties in the cell wall of live mycobacteria are consistent with structural reports in the literature, which were obtained via degradative analysis. Most importantly, using intact cells, it was possible to directly demonstrate the effects of Ethambutol on the mycobacterial cell wall polysaccharides, characterize the effects of embB gene knockout in the \textit{M. smegmatis} \textbf{DeltaembB} mutant, and observe differences in the cell wall structures of two mycobacterial species (\textit{M. bovis} BCG and \textit{M. smegmatis}). Herein, we show that HR-MAS NMR is a powerful, rapid, non-destructive technique to monitor changes in the complex, carbohydrate rich cell wall of live mycobacterial cells.—Authors’ Abstract


\textit{Mycobacterium tuberculosis} resides within the phagocytes of its host. It ensures its continued survival through arresting the normal maturation of its phagosome, which is retained within the early endosomal system of the macrophage. Although individual bacterial components have been shown to modulate phagosome biogenesis, the mechanism(s) active in live, intact bacteria remain elusive. We have developed a genetic screen that facilitates the isolation of mutants defective in arresting the maturation of their phagosomes. Macrophages were incubated with iron-dextran that was chased into lysosomes. The cells were subsequently infected with \textit{M. tuberculosis} from a library of transposon-mutagenized bacteria. After four rounds of enrichment, the majority of mutants isolated were unable to prevent acidification of their phagosomes and were attenuated for intracellular survival. The genes affected range in function from those with no known homologues to putative transporters and lipid synthesis enzymes. Further characterization of these bacteria is needed. In addition to clarifying the processes active in modulation of phagosome biogenesis by \textit{M. tuberculosis}, this screen may be applicable to other pathogens that restrict the maturation of their phagosome.—Authors’ Abstract

\textbf{Sarkola, A., Makinen, J., Marjamaki, M., Marttila, H. J., Viljanen, M. K., and Soini, H.} Prospective evaluation of the GenoType assay for routine identification (1) establish a precise structural model of the molecule and (2) decipher the structure/function relationships. In recent years, we have focused on the two domains essential for LAM biologic activities: the mannosylphosphatidylinositol anchor and the caps. We review here the recent procedures developed for the structural analysis of these domains.—Authors’ Abstract

In order to evaluate the proficiency of the GenoType Mycobacteria strip hybridization assay (Hain Lifescience, Nehren, Germany) for the routine identification of mycobacteria, the assay was used to identify 178 clinical isolates during a 6-month prospective study. The GenoType results were compared to the identification results obtained with AccuProbe (GenProbe, San Diego, CA, USA) or 16S rDNA sequencing, and an overall agreement of 89.3% between GenoType and the two reference methods was reached. The GenoType assay is, thus, a rapid and reliable method for the identification of clinically important mycobacteria, and it is well suited for use in a routine laboratory.—Authors’ Abstract


We have initiated comparative genomic analysis of Mycobacterium avium subspecies by DNA microarray, uncovering 14 large sequence polymorphisms (LSPs) comprising over 700 kb that distinguish M. avium subsp. avium from M. avium subsp. paratuberculosis. Genes predicted to encode metabolic pathways were overrepresented in the LSPs, and analysis revealed a polymorphism within the mycobactin biosynthesis operon that potentially explains the in vitro mycobactin dependence of M. avium subsp. paratuberculosis.—Authors’ Abstract


Current knowledge on the structure of lipoarabinomannan (LAM) has resulted primarily from detailed studies on a few selected laboratory strains of Mycobacterium tuberculosis, Mycobacterium bovis BCG, and Mycobacterium smegmatis. Our previous work was the first to report on the salient structural features of M. tuberculosi clinical isolates and demonstrated significant structural variations. A prime effort is to correlate a particular structural characteristic with observed differences in eliciting an immunobiological response, especially in the context of CD1-restricted presentation of LAM to T cells. T cell clones derived from the cutaneous lesions of leprosy patients have been shown to recognize specifically LAM from Mycobacterium leprae and not from M. tuberculosis Erdman or H37Rv. Herein we provide further fine structural data on LAM from M. leprae (LepLAM) and a tuberculosis clinical isolate, CSU20 (CSU20LAM), which was unexpectedly recognized by the supposedly LepLAM-specific CD1-restricted T cell clones. In comparison with the de facto laboratory LAM standard from M. tuberculosis H37Rv (RvLAM), LepLAM derived from in vivo grown M. leprae is apparently simpler in its arabinan architecture with a high degree of exposed, non-mannose-capped termini. On the other hand, CSU20, an ethambutol-resistant clinical isolate, makes a vastly heterogeneous population of LAM ranging from rather small and non-mannose-capped to full-length and fully capped variants. LepLAM and CSU20LAM contain a higher level of succinylation than RvLAM, which, in the context of truncated or less elaborated arabinan, may contribute to selective recognition by T cells. LAM from all species could be resolved into discrete forms by isoelectric focusing based apparently on their arabinan heterogeneity. In the light of our current and more recent findings, we reason that all immunobiological data should be cautiously interpreted and that the actual LAM variants that may be present in vivo during infection and pathogenesis need to be taken into consideration.—Authors’ Abstract

Recently the sequence of the Mycobacterium leprae chromosome, the only known obligate intracellular mycobacterium, was completed. It has a dramatic reduction in functional genes, with a coding capacity of only 49.5%, the lowest one so far observed among bacterial genomes. The leprosy bacillus seems to preserve a minimal set of genes that allows its survival in the host. The identification of genes that are actually expressed by the bacterium is of high significance in the context of mycobacterial pathogenesis. In this current study, a proteomic approach was undertaken to identify the proteins present in the soluble/cytosol and membrane subcellular fractions obtained from armadillo derived M. leprae. Proteins from each fraction were separated by two-dimensional gel electrophoresis (2-DE) and identified by mass spectrometry. A total of 147 protein spots were identified from 2-DE patterns and shown to comprise products of 44 different genes, twenty eight of them corresponding to new proteins. Additionally, two highly basic proteins (with pI >10.0) were isolated by heparin affinity chromatography and identified by N-terminal sequencing. This study constitutes the first application of proteomics to a host-derived Mycobacterium.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 565.


We investigated how Mycobacterium tuberculosis responded to a reduced oxygen tension in terms of its pathogenicity and gene expression by growing cells under either aerobic or low-oxygen conditions in chemostat culture. The chemostat enabled us to control and vary the oxygen tension independently of other environmental parameters, so that true cause-and-effect relationships of reduced oxygen availability could be established. Cells grown under low oxygen were more pathogenic for guinea pigs than those grown aerobically. The effect of reduced oxygen on global gene expression was determined using DNA microarray. Spearman rank correlation confirmed that microarray expression profiles were highly reproducible between repeat cultures. Using microarray analysis we have identified genes that respond to a low-oxygen environment without the influence of other parameters such as nutrient depletion. Some of these genes appear to be involved in the biosynthesis of cell wall precursors and their induction may have contributed to increased
infectivity in the guinea pig. This study has shown that a combination of chemostat culture and microarray presents a biologically robust and statistically reliable experimental approach for studying the effect of relevant and specific environmental stimuli on mycobacterial virulence and gene expression.—Authors’ Abstract


Tuberculosis caused by mycobacteria, mainly Mycobacterium tuberculosis, is a major infectious disease of the respiratory system. An early diagnosis followed by chemotherapy is the major control strategy. In an effort to identify the antigens suitable for immunodiagnosis and vaccines, the proteins secreted in a culture medium from the M. tuberculosis K-strain, which is the most prevalent among the clinical isolates in Korea and belongs to the Beijing family, were analyzed by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and compared with those from the M. tuberculosis H37Rv and CDC1551 strains. Eight proteins, Rv0652, Rv1636, Rv2818c, Rv3369, Rv3865, Rv0566c, MT3304, and Rv3160, were identified by matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) or liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) and found to be relatively abundant in the culture medium from the M. tuberculosis K-strain but less so from the CDC1551 or H37Rv strains. In addition, Rv3874 (CFP-10), Rv-0560c and Rv3648c, which were expressed increasingly in the K and CDC1551 strains, were also identified using the same proteomics technology. All proteins were prepared by molecular cloning, expression in Escherichia coli led to its localization in inclusion bodies and subsequent refolding using dialysis after its extraction from the same resulted in extensive precipitation. Therefore, an on-column refolding strategy was used, after which the protein was found to be in the soluble form. CD spectrum of the recombinant protein displayed predominantly alpha helical content (81%) which matched significantly with in silico and web-based secondary structure predictions. Furthermore, fluorescence emission spectra revealed that aromatic amino acids are buried inside the protein, which are exposed to aqueous environment under 8M urea. These results, for the first time, provide evidence on the structural features of PPE family protein which, viewed with its reported immunodominant charac-


About 10% of the coding sequence of Mycobacterium tuberculosis corresponds to hitherto unknown members of the PE and PPE protein families which display significant sequence and length variation at their C-terminal region. It has been suggested that this could possibly represent a rich source of antigenic variation within the pathogen. We describe the purification and biophysical characterization of the recombinant PPE protein coded by hypothetical ORF Rv2430c, a member of the PPE gene family that was earlier shown to induce a strong B cell response. Expression of the recombinant PPE protein in Escherichia coli led to its localization in inclusion bodies and subsequent refolding using dialysis after its extraction from the same resulted in extensive precipitation. Therefore, an on-column refolding strategy was used, after which the protein was found to be in the soluble form. CD spectrum of the recombinant protein displayed predominantly alpha helical content (81%) which matched significantly with in silico and web-based secondary structure predictions. Furthermore, fluorescence emission spectra revealed that aromatic amino acids are buried inside the protein, which are exposed to aqueous environment under 8M urea. These results, for the first time, provide evidence on the structural features of PPE family protein which, viewed with its reported immunodominant charac-
characteristics, have implications for other proteins of the PE/PPE family.—Authors’ Abstract


The majority of individuals infected with TB develop a latent infection, in which organisms survive within the body while evading the host immune system. Such persistent bacilli are capable of surviving several months of combinatorial antibiotic treatment. Evidence suggests that stationary phase bacteria adapt to increase their tolerance to environmental stresses. We have developed a unique *in vitro* model of dormancy based on the characterization of a single, large volume fermenter culture of *M. tuberculosis*, as it adapts to stationary phase. Cells are maintained in controlled and defined aerobic conditions (50% dissolved oxygen tension), using probes that measure dissolved oxygen tension, temperature, and pH. Microarray analysis has been used in conjunction with viability and nutrient depletion assays to dissect differential gene expression. Following exponential phase growth the gradual depletion of glucose/glycerol resulted in a small population of survivors that were characterized for periods in excess of 100 days. Bacilli adapting to nutrient depletion displayed characteristics associated with persistence *in vivo*, including entry into a non-replicative state and the up-regulation of genes involved in beta-oxidation of fatty acids and virulence. A reduced population of non-replicating bacilli went on to adapt sufficiently to re-initiate cellular division.—Authors’ Abstract


Tuberculosis (TB) is characterized by lifetime persistence of *Mycobacterium tuberculosis*. Despite the induction of a vigorous host immune response that curtails disease progression in the majority of cases, the organism is not eliminated. Subsequent immunosuppression can lead to reactivation after a prolonged period of clinical latency. Thus, while it is clear that protective immune mechanisms are engaged during *M. tuberculosis* infection, it also appears that the pathogen has evolved effective countermechanisms. Genetic studies with animal infection models and with patients have revealed a key role for the cytokine gamma interferon (IFN-gamma) in resistance to TB. IFN-gamma activates a large number of antimicrobial pathways. Three of these IFN-gamma-dependent mechanisms have been implicated in defense against *M. tuberculosis*: inducible nitric oxide synthase (iNOS), phagosome oxidase (phox), and the phagosome-associated GTPase LRG-47. In order to identify bacterial genes that provide protection against specific host immune pathways, we have developed the strategy of differential signature-tagged transposon mutagenesis. Using this approach we have identified three *M. tuberculosis* genes that are essential for progressive *M. tuberculosis* growth and rapid lethality in iNOS-deficient mice but not in IFN-gamma-deficient mice. We propose that these genes are involved in pathways that allow *M. tuberculosis* to counter IFN-gamma-dependent immune mechanisms other than iNOS.—Authors’ Abstract


*Mycobacterium tuberculosis* residing within pulmonary granulomas and cavities represents an important reservoir of persistent organisms during human latent tuberculosis infection. We present a novel *in vivo* model of tuberculosis involving the encapsulation of bacilli in semidiffusible hollow
fibers that are implanted subcutaneously into mice. Granulomatous lesions develop around these hollow fibers, and in this microenvironment, the organisms demonstrate an altered physiologic state characterized by stationary-state colony-forming unit counts and decreased metabolic activity. Moreover, these organisms show an antimicrobial susceptibility pattern similar to persistent bacilli in current models of tuberculosis chemotherapy in that they are more susceptible to the sterilizing drug, rifampin, than to the bactericidal drug isoniazid. We used this model of extracellular persistence within host granulomas to study both gene expression patterns and mutant survival patterns. Our results demonstrate induction of dosR (Rv3133c) and 20 other members of the DosR regulon believed to mediate the transition into dormancy, and that rel(Mtb) is required for Mycobacterium tuberculosis survival during extracellular persistence within host granulomas. Interestingly, the dormancy phenotype of extracellular M. tuberculosis within host granulomas appears to be immune mediated and interferon-gamma dependent.—Authors’ Abstract


Mycobacterium tuberculosis infects one-third of the world’s population and causes two million deaths annually. Its intracellular residence raises the possibility that bacterial nucleic acids might interact with key host proteins and contribute to the pathophysiology of infection. To test this hypothesis, we searched for motifs closely resembling eukaryotic transcription factor binding sites in the M. tuberculosis H37Rv genome and found activator protein-2 and zinc finger protein-5 binding motifs in a 36-nucleotide repetitive mycobacterial DNA sequence (RPT1). RPT1 bound specifically to nuclear extract proteins from U937, A549, and HeLa cells in electrophoretic mobility shift assays but not to activator protein-2. Several nuclear and cytosolic proteins showing at least partial binding specificity for RPT1 were isolated from U937 and A549 cells by pull-down assays, including Ku70 (DNA-dependent protein kinase subunit) and poly(ADP-ribose) polymerase-1. RPT1 also specifically activated DNA-dependent protein phosphorylation. These results suggest that mycobacterial nucleic acid fragments may interact specifically with eukaryotic regulatory proteins which might contribute to bacterial life-cycle maintenance.—Authors’ Abstract

Although the deletion of RD1 is likely correlated to attenuation from virulence for members of the Mycobacterium tuberculosis (MTB) complex, the reasons for this phenotype remain to be fully explained. As genomic variation is responsible for at least a component of variability in gene expression, we looked to the in vitro global expression profile of the RD1 artificial knockout from M. tuberculosis H37Rv (H37Rv:deltaRD1) for clues to elucidate its phenotypic shift towards attenuation. By comparing the transcriptome of H37Rv:deltaRD1 to that of virulent H37Rv, 15 regulated genes located in nine different regions outside of RD1 have been identified, capturing an effect of RD1’s deletion on the rest of the genome. To assess whether these regulations are characteristic of attenuated MTB in general, expression profiles of natural RD1 mutants (BCG Russia, BCG Pasteur, and M. microti) as well as the “avirulent” M. tuberculosis H37Ra, whose RD1 region is genomically intact, were obtained. Results indicate that attenuated strains lack the expression of RD1 genes including cfp10 and esat6, whether through deletion or reduced expression. Furthermore, comparative transcriptomics reveals the concurrent down-regulation of several gene neighborhoods beyond RD1. The potential relevance of these other expression changes towards MTB virulence is discussed.—Authors’ Abstract


The response of Mycobacterium tuberculosis to six anti-microbial agents was determined by microarray analysis in an attempt to define mechanisms of innate resistance in M. tuberculosis. The gene expression profiles of M. tuberculosis after treatment at the minimal inhibitory concentration (MIC) for 4 hr with isoniazid, isoxyl, tetrahydrolipstatin, SR1#221, SR1#967 and SR1#9190 were compared to untreated M. tuberculosis. A common response to drug exposure was defined, and this expression profile overlapped with a number of other mycobacterial stress responses recently identified by microarray analysis. Compound-specific responses were also distinguished including a number of putative transcriptional regulators and translocation-related genes. These genes may contribute to the intrinsic resistance of M. tuberculosis to anti-microbial compounds. Further investigation into these mechanisms may elucidate novel pathways contributing to mycobacterial drug resistance and influence anti-mycobacterial drug development strategies.—Authors’ Abstract

Experimental Infections


The control of Mycobacterium tuberculosis infection is dependent on the development of an adaptive immune response, which is mediated by granulomas. The granuloma is a dynamic structure that forms in the lung and consists primarily of macrophages and lymphocytes. For this structure to be effective in containment of the bacillus, it must develop in an organized and timely manner. The formation of the granuloma is dependent on recruitment of activated cells through adhesion molecules and chemokines. M. tuberculosis infection causes an increase in the expression of beta-chemokines CCL3, CCL4, and CCL5, and their receptor CCR5, in the lungs. In this study, we demonstrate that CCR5-transgenic knockout mice were capable of recruiting immune cells to the lung to form granulomas. CCR5(−/−) mice successfully induced a Th1 response and controlled infection. Unexpectedly, M. tuberculosis infection in these mice resulted in greater numbers of lymphocytes migrating to the lung and higher levels of many inflammatory cytokines, compared with wild-type mice,
without apparent long-term detrimental effects. In the absence of CCR5, there were more dendritic cells in the lung-draining lymph nodes and more primed T lymphocytes in these mice. Bacterial numbers in the lymph nodes were also higher in CCR5(−/−) mice. Therefore, CCR5 may play a role in the migration of dendritic cells to and from the lymph nodes during *M. tuberculosis* infection.—Authors’ Abstract


In the past decade, while the global tuberculosis (TB) epidemic has continued to devastate mankind, considerable progress has nevertheless been made in the development of new and improved vaccines for this ancient disease. Recombinant bacillus Calmette-Guerin strains, DNA-based vaccines, live attenuated *Mycobacterium tuberculosis* vaccines and subunit vaccines formulated with novel adjuvants have shown promise in preclinical animal challenge models. Three of these vaccines are being evaluated at present in human clinical studies, and several other vaccine preparations are being targeted for clinical trials in the near future. Although the preclinical characterisation and testing of new TB vaccines has clearly led to exciting new findings, complex regulatory and clinical trial design issues remain as a challenge to TB vaccine development. This report reviews some of the exciting advances in TB research that have led to the development of new TB vaccines, and addresses the unique regulatory and clinical issues associated with the testing of novel anti-TB preparations in human populations.—Authors’ Abstract


*Mycobacterium microti*, the vole bacillus, which was used as a live vaccine against tuberculosis until the 1970s, confers the same protection in humans as does *Mycobacterium bovis* bacille Calmette-Guerin (BCG). However, because the efficacy of the BCG vaccine varies considerably, we have tried to develop a better vaccine by reintroducing into *M. microti* the complete region of difference 1 (RD1), which is required for secretion of the potent T cell antigens early secreted antigen target (ESAT)-6 and culture filtrate protein (CFP)-10. The resultant recombinant strain, *M. microti* OV254::RD1-2F9, induced specific ESAT-6 and CFP-10 immune responses in mice with CD8(+) T lymphocytes that had strong expression of the CD44(hi) activation marker. This vaccine also displayed better efficacy against disseminated disease in the mouse and the guinea pig models of tuberculosis than was seen in animals vaccinated with *M. microti* alone or with BCG. The *M. microti* OV254::RD1-2F9 vaccine was less virulent and persistent in mice and than was BCG::RD1-2F9 may represent a safer alternative to BCG::RD1-2F9.—Authors’ Abstract


The only available vaccine against tuberculosis (TB) is Bacillus Calmette-Guérin (BCG) whose efficacy in preventing pulmonary tuberculosis is however controversial. Here, we show that BCG infection of monocytes causes their differentiation into mature dendritic cells (DCs) lacking CD1 molecules expression, coupled with suboptimal up-regulation of HLA class II, CD80 and CD40 molecules and a marked unresponsiveness to lipopolysaccharide stimula-
tion. In addition, alloreactive naïve T lymphocytes primed by these subverted DCs did not undergo defined functional polarization, as witnessed by their inability to produce IFN-gamma. Since efficient antigen presentation and IFN-gamma production by mycobacterial-specific T lymphocytes are required for protection against *Mycobacterium tuberculosis*, our data might provide additional explanation for the low efficacy of BCG vaccination.—Authors’ Abstract


Recombinant mycobacteria expressing *Mycobacterium tuberculosis* extracellular proteins are leading candidates for new vaccines against tuberculosis and other mycobacterial diseases, and important tools both in antimycobacterial drug development and basic research in mycobacterial pathogenesis. Recombinant mycobacteria that stably overexpress and secrete major extracellular proteins of *M. tuberculosis* in native form on plasmids pSMT3 and pNBV1 were previously constructed by the authors. To enhance the versatility of this plasmid-based approach for mycobacterial protein expression, the *Escherichia coli* mycobacteria shuttle plasmid pGB9 was modified to accommodate mycobacterial genes expressed from their endogenous promoters. Previous studies showed that the modified plasmid, designated pGB9.2, derived from the cryptic *Mycobacterium fortuitum* plasmid pMF1, was present at a low copy number in both *E. coli* and mycobacteria, and expression of recombinant *M. tuberculosis* proteins was found to be at levels paralleling its copy number, that is, approximating their endogenous levels. Plasmid pGB9.2 was compatible with the shuttle vectors pSMT3 and pNBV1 and in combination with them it simultaneously expressed the *M. tuberculosis* 30 kDa extracellular protein FbpB. Plasmid pGB9.2 was stably maintained in the absence of selective pressure in three mycobacterial species: *Mycobacterium bovis* BCG, *M. tuberculosis* and *M. smegmatis*. Plasmid pGB9.2 was found to be self-transmissible between both fast- and slow-growing mycobacteria, but not from mycobacteria to *E. coli* or between *E. coli* strains. The combination of two compatible plasmids in one BCG strain allows expression of recombinant mycobacterial proteins at different levels, a potentially important factor in optimizing vaccine potency.—Authors’ Abstract


In September 2000, recognizing the effect of communicable diseases as obstacles to development in poorer countries, the European Commission assembled a special round table on “accelerated action targeted at major communicable diseases within the context of poverty reduction.” The three major communicable diseases discussed were tuberculosis (TB), malaria and HIV. One outcome of this discussion was a workshop examining issues related to the fight against TB in Africa, which took place in Gorée, Sénégal, in May 2001. The timing was propitious, as new vaccines for TB (recombinant MVA and BCG, and adjuvanated recombinant fusion proteins or peptide constructs), are just beginning to enter human clinical trials. All but the last of these have shown promise in animal models, up to and including non-human primates, and all are strongly immunogenic and apparently safe. Humans trials for safety and efficacy are thus the logical next step. This review summarizes recent advances in tuberculosis vaccine development, with a special emphasis on issues raised at the Gorée meeting about testing and deploying new generation vaccines in TB-endemic areas such as Africa.—Authors’ Abstract

Immunization with plasmid DNA vectors represents a promising new approach to vaccination. It has been shown to elicit humoral and cellular immunity and protection in various infection models. Here, we assessed the immunogenicity and protective efficacy of a DNA vaccine vector encoding the antigen 85A (Ag85A) of *Mycobacterium tuberculosis*. Since intramuscular (i.m.) immunization with naked DNA requires considerable amounts of DNA in order to be effective, we evaluated a strategy to reduce the amount of DNA needed. To this end, we used Ag85A DNA adsorbed onto cationic poly(DL-lactide-co-glycolide) (PLG) microparticles and observed similar levels of protection against aerosol challenge in mice using doses of PLG-DNA two orders of magnitude lower than with naked DNA itself.—Authors’ Abstract


The present study describes a novel and simple vaccination strategy that involves the culturing of live *Mycobacterium tuberculosis* and *Salmonella typhimurium* in syngeneic, allogeneic, and xenogeneic macrophages, followed by drug treatment and gamma irradiation, to kill the bacteria. Notable observations were that the lymphocytes obtained from the vaccinated mice proliferated and secreted mainly interferon- gamma and IgG2a, but not interleukin-4 and IgG1. The enumeration of viability of *M. tuberculosis* indicated a significant level of protection in the vaccinated mice after challenge with live *M. tuberculosis*. This vaccination strategy worked successfully for tuberculosis but also showed a significant decrease in mortality of mice challenged with live *S. typhimurium*.—Authors’ Abstract


Key Ags of *Mycobacterium tuberculosis* initially identified in the context of host responses in healthy purified protein derivative-positive donors and infected C57BL/6 mice were prioritized for the development of a subunit vaccine against tuberculosis. Our lead construct, Mtb72F, codes for a 72-kDa polyprotein genetically linked in tandem in the linear order Mt32(C)-Mt39-Mt32(N). Immunization of C57BL/6 mice with Mtb72F DNA resulted in the generation of IFN-gamma responses directed against the first two components of the polyprotein and a strong CD8(+) T cell response directed exclusively against Mt32(C). In contrast, immunization of mice with Mtb72F protein formulated in the adjuvant AS02A resulted in the elicitation of a moderate IFN-gamma response and a weak CD8(+) T cell response to Mt32c. However, immunization with a formulation of Mtb72F protein in AS01B adjuvant generated a comprehensive and robust immune response, resulting in the elicitation of strong IFN-gamma and Ab responses encompassing all three components of the polyprotein vaccine and a strong CD8(+) response directed against the same Mt32(C) epitope identified by DNA immunization. All three forms of Mtb72F immunization resulted in the protection of C57BL/6 mice against aerosol challenge with a virulent strain of *M. tuberculosis*. Most importantly, immunization of guinea pigs with Mtb72F, delivered either as DNA or as a rAg-based vaccine, resulted in prolonged survival (>1 year) after aerosol challenge with virulent *M. tuberculosis* comparable to bacillus Calmette-Guérin immunization. Mtb72F in AS02A formulation is currently in phase I clinical trial, making it the first recombinant tuberculosis vaccine to be tested in humans.—Authors’ Abstract

Cell-mediated immune responses are crucial in the protection against tuberculosis. In this study, we constructed epitope DNA vaccines (p3-M-38) encoding cytotoxic T lymphocyte (CTL) epitopes of MPT64 and 38 kDa proteins of Mycobacterium tuberculosis. In order to observe the influence of spacer sequence (Ala-Ala-Tyr) or ubiquitin (UbGR) on the efficacy of the two CTL epitopes, we also constructed DNA vaccines, p3-M-S(spacer)-38, p3-Ub (UbGR)-M-S-38 and p3-Ub-M-38. The immune responses elicited by the four DNA vaccines were tested in C57BL/6 (H-2b) mice. The cytotoxicity of T cells was detected by LDH-release method and by enzyme-linked immunospot assay for epitope-specific cells secreting interferon-gamma. The results showed that DNA immunization with p3-M-38 vaccine could induce epitope-specific CD8+ CTL response and that the spacer sequence (AAY) only enhanced M epitope presentation. The protein-targeting sequence (UbGR) enhanced the immunogenicity of the two epitopes. The finding that defined spacer sequences at C-terminus and protein-targeting degradation modulated the immune response of epitope string DNA vaccines will be of importance for the further development of multi-epitope DNA vaccines against tuberculosis.—Authors’ Abstract

**Epidemiology and Prevention**


BACKGROUND: Not every leprosy patient is equally effective in transmitting Mycobacterium leprae. We studied the spatial distribution of infection (using seropositivity as a marker) in the population to identify which disease characteristics of leprosy patients are important in transmission. METHODS: Clinical data and blood samples for anti-\textit{M. leprae} ELISA were collected during a cross-sectional survey on five Indonesian islands highly endemic for leprosy. A geographic information system (GIS) was used to define contacts of patients. We investigated spatial clustering of patients and seropositive people and used logistic regression to determine risk factors for seropositivity. RESULTS: Of the 3986 people examined for leprosy, 3271 gave blood. Seroprevalence varied between islands (1.7–8.7%) and correlated significantly with leprosy prevalence. Five clusters of patients and two clusters of seropositives were detected. In multivariate analysis, seropositivity significantly differed by leprosy status, age, sex, and island. Serological status of patients appeared to be the best discriminator of contact groups with higher seroprevalence: contacts of seropositive patients had an adjusted odds ratio (aOR) of 1.75 (95% CI 0.92–3.31). This increased seroprevalence was strongest for contact groups living ≤75 m of two seropositive patients (aOR = 3.07; 95% CI 1.74–5.42). CONCLUSIONS: In this highly endemic area for leprosy, not only household contacts of seropositive patients, but also people living in the vicinity of a seropositive patient were more likely to harbour antibodies against \textit{M. leprae}. Through measuring the serological status of patients and using a broader definition of contacts, higher risk groups can be more specifically identified.—Authors’ Abstract


Introduction and purpose: Some authors demonstrated the possibility of the armadillos, Dasypus novemcinctus, being an envi-
An epidemiologic survey was done to check the correlation between the human contact with armadillos and the incidence of leprosy. It discusses some features that could be involved in the dynamic process of the leprosy development. The objective of this research is to check the frequency of cases of leprosy contacts with armadillos and also the interhuman contact before their diagnosis to establish the possibility of the *M. leprae* transmission to the human being through the contact with armadillos.

**Methods:** One hundred and seven leprosy patients were surveyed (leprosy patients that had finished the MTD treatment) who lived in the Pedro Fontes Colony-Hospital, in Cariacica, Espirito Santo State, Brazil, 29 leprosy patients and 173 non leprosy patients from a dermatology service of the city of Vitória, Brazil. The survey included data about the armadillo meat consumption before leprosy diagnosis, the existence of known cases and/or familial leprosy cases. The data were analyzed by Qui-square test, correlation and Exact Fischer Test.

**Results:** 90.4% of the leprosy patients or cured leprosy patients had once eaten armadillo meat before their leprosy diagnosis, while 15% of the non leprosy patients had already eaten armadillo meat. In a group without leprosy contact before the diagnosis, 96.1% ate armadillo meat, and 3.9% didn’t eat. This study supposes a possible source of the *M. leprae* by the armadillo meat consumption, mainly, in a leprosy patients without previous leprosy contact.—Hansenologia Internationalis


Although the prevalence of leprosy has declined over the years, there is no evidence that incidence rates are falling. A method of early detection of those people prone to develop the most infectious form of leprosy would contribute to breaking the chain of transmission. Prophylactic treatment of serologically identified high-risk contacts of incident patients should be an operationally feasible approach for routine control programs. In addition, classification of high-risk household contacts will allow control program resources to be more focused. In this prospective study, we examined the ability of serology used for the detection of antibodies to phenolic glycolipid I of *Mycobacterium leprae* to identify those household contacts of multibacillary leprosy patients who had the highest risk of developing leprosy. After the start of multidrug therapy for the index case, a new case of leprosy developed in one in seven of the 178 households studied. In households where new cases appeared, the seropositivity rates were significantly higher (p <0.001) than those in households without new cases. Seropositive household contacts had a significantly higher risk of developing leprosy (relative hazard adjusted for age and sex [aRH], 7.2), notably multibacillary leprosy (aRH = 24), than seronegative contacts.—Authors’ Abstract


Between 1986 and 2002, a total of 28 new leprosy cases were notified to the Kimberly Public Health Unit in Western Australia, Australia. At diagnosis, the patients were aged 8–63 years. In several recent cases, diagnosis was delayed despite multiple presentations to primary health care staff and medical specialists. Eleven patients (39%) had multibacillary disease, and can transmit the disease. The need for the proper management of leprosy patients and to control the increasing population movement in and out of leprosy-endemic areas to prevent leprosy transmission to other parts of Australia is discussed.—Tropical Diseases Bulletin


See Current Literature, General and Historical, p. 511.
Deepak, S. Answering the rehabilitation needs of leprosy-affected persons in integrated setting through primary health care services and community-based rehabilitation. Indian J. Lepr. 75(2) (2003) 127–142.

This article aims to discuss the strategies for answering the rehabilitation needs of persons with leprosy-related disabilities in integrated settings through primary health care (PHC) services and community-based rehabilitation (CBR). While the provision of rehabilitation services through the PHC system remains problematic in most developing countries, the article concludes that CBR programs have the potential for rehabilitation of leprosy-affected persons in integrated settings. However, the limited coverage of CBR programs may pose an obstacle to such an approach. The author suggests the use of existing specific rehabilitation infrastructures meant only for leprosy-affected persons for initiating, sustaining and extending the CBR coverage to the surrounding communities. At the same time, the author asks for support and strengthening of organizations of leprosy-affected persons, promoting their active involvement in all rehabilitation processes.—Authors’ Abstract


Leprosy is considered to cause more social than medical problems. The present study focussed on this aspect in order to investigate the level of awareness among people—about their attitude towards the disease and the afflicted. The results are based on interviews with 104 persons in Delhi. The sample data revealed that the level of knowledge of leprosy was inadequate. The cause of the disease was known to 44.2% of those interviewed, while 31.7% were completely ignorant; 6.7% believed it to be the consequence of an individual’s past misdeeds, and 1.9% believed it to have been caused by divine curse. 63.1% were aware that the disease is curable. 73.1% of the persons interviewed sympathised with leprosy-afflicted beggars. 61.5% favored leprosy patients to stay with their families and within their communities. 67.3% felt that the cured could marry, while 25% felt that the leprosy-afflicted should stay in leprosy colonies away from the society. 54.8% were reluctant to employ the leprosy-afflicted as domestic help, and 31.7% were reluctant to establish matrimonial relationship with a family having a leprosy-afflicted person. The data call for intensification of public awareness regarding the aetiology of leprosy. Positive and scientific information should be disseminated to minimize the social prejudices associated with the disease.—Authors’ Abstract


A 56-year-old male was transferred to our centre because of a relapse of leprosy neuritis in the hands. We found that the patient had received a posterior tibialis tendon transfer for correction of his left dropped foot 40 years previously. On examination active dorsiflexion of the left ankle joint was close to 0 degrees with grade 4 power of dorsiflexion, and the plantar flexion was about 35 degrees. Walking gait was almost normal. There were some scars on the planter surface of the left metatarsal area; but with the continuous use of a soft dressing pad under the middle part of the sole, plantar ulceration has been avoided for many years even with active daily activities of the patient. The patient is very satisfied with the operative results.—Authors’ Abstract

The clinical and epidemiological characteristics of 17 patients diagnosed with *Mycobacterium kansasii* pneumonia within a limited geographical region over a period of 10 years are described. An in-depth evaluation of the innate and adaptive immune systems was performed for five available patients. A comparison was made of the genetic fingerprint patterns of the isolates obtained by restriction fragment length polymorphism (RFLP) analysis, with the major polymorphic tandem repeat (MPTR) as a probe. Predisposing factors consisted of smoking, airway abnormalities, substance abuse, diabetes or poor general condition, but in two patients no risk factor was identified. In the five patients tested, no abnormalities or deficiencies were detected in the innate or adaptive type-1 immunity. All *M. kansasii* isolates had identical MPTR RFLP patterns, although no epidemiological connection could be established, and these were identical to those of clinical isolates from Australian patients. These data do not support the theory that defects in the innate or adaptive type-1 immunity have a role in the pathogenesis of invasive *M. kansasii* infections. The identical fingerprint patterns of the isolates suggested the existence of a virulent strain of *M. kansasii*.—Authors’ Abstract


Data from 1700 patients living in southern Benin were collected at the Centre Sanitaire et Nutritionnel Gbemoten, Zagnanado, Benin, from 1997 through 2001. In the Zou region in 1999, Buruli ulcer (BU) had a higher detection rate (2.15/100,000) than leprosy (13.4/100,000) and tuberculosis (20.0/100,000). More than 13% of the patients had osteomyelitis. Delay in seeking treatment declined from 4 months in 1989 to 1 month in 2001, and median hospitalization time decreased from 9 months in 1989 to 1 month in 2001. This reduction is attributed, in part, to implementing an international cooperation program, creating a national BU program, and making advances in patient care.—Emerging Infectious Diseases


Five *Mycobacterium tuberculosis* complex isolates in California were identified as *M. africanum* by spoligotyping, single nucleotide polymorphisms, a deletion mutation, and phenotypic traits, confirming it as a cause of tuberculosis in the United States. Three of the five patients from whom *M. africanum* was isolated had lived in Africa. —Authors’ Abstract


*Mycobacterium ulcerans* gives rise to severe skin ulceration that can be associated with considerable illness. The cost of diagnosis, treatment, and lost income has never been assessed in Australia. A survey of 26 confirmed cases of the disease in Victoria was undertaken. Data were collected on demographic details, diagnostic tests, treatment, time off work, and travel to obtain treatment. All costs are reported in Australian dollars in 1997–1998 prices. The cost varies considerably with disease severity. For mild cases, the average direct cost is
6803 Australian dollars, and for severe cases 27,681 Australian dollars. Hospitalization accounts for 61% to 90% of costs, and indirect costs amount to 24% of the total per case. *M. ulcerans* can be an expensive disease to diagnose and treat. Costs can be reduced by early diagnosis and definitive treatment. Research is needed to find cost-effective therapies for this disease.—Authors’ Abstract


This study reports a potential role that fish may play in the transmission of *Mycobacterium ulcerans* disease (Buruli ulcer). Fish found positive for *M. ulcerans* DNA all appear to feed on insects or plankton and are believed to concentrate *M. ulcerans* from this usual food source. These observations provide additional data supporting our previous hypothesis on sources of *M. ulcerans* and modes of transmission.—Authors’ Abstract


*Mycobacterium avium* complex (MAC) is ubiquitous. It is found in various freshwater and saltwater sources around the world, including hot water pipes. Although the organism was identified in the 1890s, its potential to cause human disease was only recognized 50 years later. Only a minority of people exposed to the organism will acquire MAC lung disease, usually those with underlying lung disease or immunosuppression. MAC may, however, cause progressive parenchymal lung disease and bronchiectasis in patients without underlying lung disease, particularly in middle-aged and elderly women. Preliminary data suggest that the interferon-gamma pathways may be deficient in elderly women with MAC lung disease. Other groups of patients who are more likely to harbor MAC in their lungs include patients with a cystic fibrosis or an abnormal alpha(1)-antiproteinase gene and patients with certain chest wall abnormalities. Treatment results continue to be disappointing, and the mortality of patients with MAC lung disease remains high. A PubMed search identified 38 reports of the treatment of MAC lung disease. Apart from the British Thoracic Society study, the only published controlled investigation, the studies published since 1994 have included a macroclide, either clarithromycin or azithromycin, usually in combination with ethambutol and a rifamycin. If success is defined as eradication of the organism without relapse over a period of several years after treatment has been discontinued, the reported treatment success rate with the macrolide containing regimens is approximately 55%. The prolonged treatment period, side effects, and possibly reinfection rather than relapse are responsible for the high failure rate.—Authors’ Abstract


The relationship between silicosis and tuberculosis is well known. Also other mycobacteria such as *Mycobacterium kansasii* often occur in association with pneumoconiosis. However, there are few reports describing an association of *M. avium*-intracellulare complex (MAC) lung disease and pneumoconiosis. The purpose of the present study is to describe clinical features of MAC respiratory infection associated with pneumoconiosis. Eleven patients with MAC respiratory infection associated with pneumoconiosis (all men, 6 with silicosis and 5 with welders’ pneumoconiosis) were collected. A determination of whether or not MAC caused pulmonary disease was made using the 1997 criteria required by the American Thoracic Society. Radiologically,
cavity formation as well as upper lung field predominance of MAC disease were observed in 8 of 11 cases (72.7%). Two of 11 patients died of respiratory failure. Our present study clearly demonstrates that clinical features of MAC respiratory infection associated with pneumoconiosis were different from MAC without underlying diseases.—Authors’ Abstract


Mycobacterium microti is the agent of tuberculosis in wild voles and has been used as a live vaccine against tuberculosis in man and cattle. To explore the M. microti genome in greater detail, we used a M. tuberculosis H37Rv genomic DNA microarray to detect gene deletions among M. microti isolates. A number of deletions were identified that correlated with those described previously (Infect. Immun. 70 (2002) 5568) but a novel M. microti deletion was also found (MiD4) which removes 5 genes that code for ESAT-6 family antigens and PE-PPE proteins. Southern blot experiments showed that this region was also deleted from M. pinnipedii, a mycobacterium isolated from seals that is closely related to M. microti. Genes encoding ESAT-6 antigens and PE-PPE proteins appear to be frequently deleted from M. microti, and the implications of this are discussed.—Authors’ Abstract


We performed a prospective, 2-year nationwide study to assess incidence and disease characteristics of suspected infections with nontuberculous mycobacteria (NTM) in children, via the Netherlands Pediatric Surveillance Unit. Data for 61 children were reported (median age, 31 months; interquartile range, 22–50 months; female sex, 37 subjects); 2 subjects had an underlying disease. Most children (53 [87%] of 61) had cervical lymph node enlargement, with abscess in 25 (47%) and fistula in 11 (21%). The estimated annual incidence of NTM infection was 77 cases per 100,000 children. In 16 children, the diagnosis was based solely on the results of skin tests with mycobacterial antigens. Cultures were performed in 36 cases and yielded mycobacteria in 27 (75%); Mycobacterium avium was isolated from 18 cultures. Children with a culture positive for mycobacteria did not differ in presentation, complications, or treatment from those whose cultures showed no growth. Thirty children underwent surgery, and chemotherapy was the single treatment in 24 (39%) of the cases. The treatment of localized NTM infection in immunocompetent children by antimycobacterial drugs should be evaluated further.—Authors’ Abstract


Dementia developed in a patient with widespread neurologic manifestations; she died within 5 months. Pathologic findings showed granulomatous inflammation with caseation necrosis, foreign body-type giant cells, and proliferative endarteritis with vascular occlusions. Broad-range polymerase chain reaction identified Mycobacterium neoaurum as the possible pathogen. Central nervous system infection by M. neoaurum may result in rapidly progressive dementia.—Authors’ Abstract

Hyon, J. Y., Joo, M. J., Hose, S., Sinha, D., Dick, J. D., and O’Brien, T. P. Comparative efficacy of topical gatifloxacin with ciprofloxacin, amikacin, and clarithromycin in the treatment of experi-
OBJECTIVE: To determine the comparative efficacy of topical gatifloxacin with ciprofloxacin, fortified amikacin, and clarithromycin against *Mycobacterium chelonae* keratitis in an animal model. METHODS: Experimental *M. chelonae* keratitis was induced via intrastromal inoculation in a rabbit model. Thirty-five rabbits were randomly divided into 5 groups and each group was treated hourly for 12 hours with topical 0.9% balanced salt solution, 0.3% gatifloxacin, 0.3% ciprofloxacin hydrochloride, a combination of topical fortified amikacin sulfate (50 mg/mL) and clarithromycin (10 mg/mL), or a triple combination of topical 0.3% gatifloxacin, fortified amikacin sulfate (50 mg/mL), and clarithromycin (10 mg/mL). Antibacterial efficacy of each regimen was determined by quantitative bacteriologic analysis. RESULTS: Treatment with 0.3% gatifloxacin or the triple combination of 0.3% gatifloxacin, fortified amikacin sulfate (50 mg/mL), and clarithromycin (10 mg/mL) reduced the number of mycobacterial organisms more significantly than the controls that were treated with a topical balanced salt solution (both p <.001). Therapy with 0.3% gatifloxacin was more effective than 0.3% ciprofloxacin alone (p <.001) and demonstrated synergy by enhancing the efficacy of the combination of fortified amikacin (50 mg/mL) and clarithromycin (10 mg/mL) (p <.001). Neither 0.3% ciprofloxacin nor the combination of fortified amikacin (50 mg/mL) and clarithromycin (10 mg/mL) demonstrated a significant difference in activity against mycobacteria compared with the topical balanced salt solution. CONCLUSION: These results suggest that topical 0.3% gatifloxacin ophthalmic solution can be a new initial treatment agent against *M. chelonae* keratitis. Clinical Relevance Topical gatifloxacin 0.3% may provide an initial alternative in therapy of *M. chelonae* keratitis.—Authors’ Abstract


We reviewed a rare breast infection occurring 4 months after nipple piercing. Clinical examination suggested carcinoma. *Mycobacterium fortuitum* was eventually isolated after surgical biopsy and debridement. Antibiotic therapy was initiated intravenously using two drugs and oral therapy was continued for 6 months. A contralateral mycobacterial lesion emerged and was excised along with a residual fibrotic nodule at the original biopsy site. When adequate sampling of a complex and suspicious breast mass is benign and initial bacterial cultures are sterile, mycobacterial infection should be considered, particularly when there is a history of previous nipple piercing procedures.—Authors’ Abstract


Reported here are two cases of *Mycobacterium malmoense* lymphadenitis that occurred in two immunocompetent children in...
Spain. To the best of our knowledge, these are the first documented cases of extrapulmonary infection by *M. malmoense* in Spain. This report serves to draw attention to this emerging nontuberculous mycobacterium that is gaining increasing recognition as a pulmonary and extrapulmonary pathogen in different countries.—Authors’ Abstract


A 48 year old patient with active Crohn’s disease presented with bilateral nodules over his lungs resembling malignant metastasis. Bronchoscopic and pathological examination of the airways and sputum did not show any malignancy. After 6 weeks *Mycobacterium xenopi* was cultured from his bronchial washings while all other cultures remained negative. Treatment was started with rifampicin, ethambutol, and clarithromycin and, after 9 months of treatment, there was an almost complete resolution of his chest radiograph.—Authors’ Abstract


SUMMARY: Guidelines recommend treating HIV-infected patients with pulmonary *Mycobacterium kansasii* infection only in the presence of multiple positive cultures and clinical and radiographic abnormalities. Some authors suggest a single positive culture warrants treatment. A systematic literature review was done to determine whether HIV-infected patients who had *M. kansasii* isolated from respiratory specimens may have an indolent infection (often referred to as colonization) not requiring treatment and to determine the utility of diagnostic criteria in distinguishing disease from indolent infection. Sixteen studies were included, with at least 573 patients: mean age 44 years; 91% male; 64% men who had sex with men; 35% injection drug users; and median CD4 lymphocyte count of 2–381 cells/μL. The median rate of indolent infection was 8%. Of the few patients who did not satisfy diagnostic criteria and were left untreated, outcomes were generally favorable. Overall, survival was longer in treated patients (mean 12 vs. 4 months). Indolent pulmonary infection with *M. kansasii* may exist in the setting of HIV, but published data do not provide adequate information to identify such patients. It is unclear whether unfulfilled diagnostic criteria necessarily imply the absence of disease in this context.—Authors’ Abstract


*Mycobacterium africanum* is thought to comprise a unique species within the *Mycobacterium tuberculosis* complex. *M. africanaum* has traditionally been identified by phenotypic criteria, occupying an intermediate position between *M. tuberculosis* and *M. bovis* according to biochemical characteristics. Although *M. africanum* isolates present near-identical sequence homology to other species of the *M. tuberculosis* complex, several studies have uncovered large genomic regions variably deleted from certain *M. africanum* isolates. To further investigate the genomic characteristics of organisms characterized as *M. africanum*, the DNA content of 12 isolates was interrogated by using Affymetrix GeneChip. Analysis revealed genomic regions of *M. tuberculosis* deleted from all isolates of putative diagnostic and biological consequence. The distribution of deleted sequences suggests that *M. africanum* subtype II isolates are situated among strains of “modern” *M. tuberculosis*. In contrast, other *M. africanum* isolates (subtype I) constitute two distinct evolutionary branches within the *M. tuberculosis* complex. To test for an association between deleted sequences and biochemical attributes used for speciation, a phenotypically diverse panel of “*M. africanum*-like” isolates from Guinea-Bissau was tested for these
deletions. These isolates clustered together within one of the M. africanum subtype I branches, irrespective of phenotype. These results indicate that convergent biochemical profiles can be independently obtained for M. tuberculosis complex members, challenging the traditional approach to M. tuberculosis complex speciation. Furthermore, the genomic results suggest a rational framework for defining M. africanum and provide tools to accurately assess its prevalence in clinical specimens.—Authors’ Abstract


Mycobacterium ulcerans disease (Buruli ulcer) is a serious ulcerating skin disease which is common in many tropical countries. Standard treatment, by extensive excision and skin grafting, is not available in rural communities where the disease is common. We evaluated the efficacy and safety of treatment with topical nitrogen oxides. Thirty-seven patients with a clinical diagnosis of Buruli ulcer caused by M. ulcerans disease were randomly assigned to one of two groups. In one group, two creams containing sodium nitrite (6%, wt/wt) or citric acid monohydrate (9%, wt/wt) were applied daily for 6 weeks, while the other group received a placebo. In the second 6 weeks, both groups received the nitrogen oxide-generating combination of creams. Treatment was continued for another 4 weeks for patients whose ulcers were not healed after 12 weeks. The ulcer surface area was monitored by weekly tracings made by assessors blinded to the treatment. In the first 6 weeks, patients on sodium nitrite and citric acid monohydrate (group I, active treatment) showed a rapid decrease in ulcer size from 28.6 ± 5.6 cm² (mean ± standard error) to 12.6 ± 3.2 cm², a decrease significantly greater than that in group II (from 15.3 ± 3.1 to 11.7 ± 3.7 cm²; p = 0.03). Five ulcers in the placebo group enlarged during this period, compared with one in the active group. In the second 6 weeks (both groups on active treatment), the rates of healing were similar for the two groups and there was a significant reduction in ulcer size in group II (previously on placebo) compared to the first 6 weeks. Yellow pigmentation of the skin, which disappeared 3 days after treatment was stopped, was the only side effect to date. We conclude that creams releasing nitrogen oxides increase the healing rate of ulcers caused by M. ulcerans infection with minimal adverse events. This is the first controlled trial of any form of therapy which demonstrates efficacy in treating this disease.—Authors’ Abstract


Mycobacterium ulcerans, which causes Buruli ulcer, was exposed to acidified nitrite or to acid alone for 10 or 20 min. Killing was rapid, and viable counts were reduced below detectable limits within 10 min of exposure to 40 mM acidified nitrite. M. ulcerans is highly susceptible to acidified nitrite in vitro.—Authors’ Abstract


A lymph node excision was performed on a 45-year-old woman with left cervical swelling. The disorder which developed after the patient had undergone oral surgery for a severe periodontal disease failed to respond to antimicrobial chemotherapy. A mycobacterial strain subsequently identified by high-performance liquid chromatography analysis of cell wall mycolic acids as Mycobacterium lentiflavum grew from the excised specimen. This case and previously published reports highlight the relevance of M. lentiflavum as an emerging causative agent of mycobacterial cervical lymphadenitis.—Authors’ Abstract

SETTING: Mycobacterium avium complex (MAC) is known to colonize the gastrointestinal tract of human immunodeficiency virus (HIV) infected patients before causing bacteremia and disseminated disease. However, the mechanism involved in the gastrointestinal colonization is not known. OBJECTIVE: To identify putative intestinal mucus receptors which serve as anchor for MAC colonization. DESIGN: C57BL/6 mouse intestinal mucus was subjected to single and two-dimensional electrophoresis and blotted on nitrocellulose membranes. MAC specific mucus proteins were identified by probing the mucus western blots with biotinylated proteins derived from M. avium strain 101 (MAC101). RESULTS: Biotinylated MAC 101 proteins recognized a 39 kDa intestinal mucus glycoprotein. The protein displaying an isoelectric point (pI) of 9.0, was found to be peridate sensitive but resistant to sialidase, heparinase I and chondroitinase ABC. The internal amino acid sequence of the 39 kDa protein displayed homology with fructose-1,6-bisphosphate aldolase B (aldolase). The proclivity between MAC adhesins and aldolase was confirmed by probing rabbit muscle aldolase with MAC proteins. Furthermore, both 25 and 31 kDa MAC adhesins, superoxide dismutase and heparin binding protein, respectively, were found to bind to aldolase. CONCLUSIONS: MAC binds to intestinal mucus aldolase, conceivably facilitating intestinal colonization of the organism.—Authors’ Abstract


We report on two toddlers suffering from Mycobacterium bohemicum lymphadenitis. Acid-fast bacilli were cultured from submandibular lymph nodes and identified by molecular methods as Mycobacterium bo- hemicum. Surgical treatment was successful and complemented by oral treatment with clarithromycin and rifampicin.—Authors’ Abstract


In this study we introduce a rapid procedure to identify Mycobacterium abscessus (types I and II) and M. chelonae using LightCycler-based analysis of the hsp65 gene. Results from 36 clinical strains were compared with hsp65 gene restriction analysis and biochemical profiles of bacilli. As all three methods yielded identical results for each isolate, this procedure offers an excellent alternative to previously established nucleic acid amplification-based techniques for the diagnosis of mycobacterial diseases.—Authors’ Abstract


INTRODUCTION: Mycobacterium haemophilum, a nontuberculous mycobacterium (NTM) that was first described in 1978, is a pathogen that can cause an array of symptoms in immunocompromised patients, predominantly cutaneous. CLINICAL PICTURE: We report our hospital’s experience with the first 3 patients diagnosed with this infection from 1994 to 2002. All were women; one had systemic lupus erythematosus (SLE), one had mycosis fungoides and the last had Sjogren’s syndrome with recurrent bacterial infections, although the specific nature of her immunocompromised state has not been defined. All were HIV negative. All 3 women presented with cutaneous lesions—the first with recurrent erythematous plaques on the limbs and back, the second with tender nodules and abscesses on the knees, and the third with papular eruptions on the cheek. TREAT-
MENT/OUTCOME: All responded to a combination of antibiotics and are presently still undergoing treatment and follow-up.

CONCLUSION: Infections caused by *M. haemophilum* occur mainly in immunocompromised patients. They can present with a variety of cutaneous manifestations, which require a high index of suspicion and coordination between the treating physician and the laboratory for diagnosis. Combination antibiotic treatment is recommended, and patients should be followed up after treatment to survey for possible relapse.—Authors’ Abstract


The possibility that the strains included within the *Mycobacterium avium* complex (MAC), but not belonging either to *M. avium* or to *Mycobacterium intracellulare*, may be members of undescribed taxa, has already been questioned by several taxonomists. A very homogeneous cluster of 12 strains characterized by identical nucleotide sequences both in the 16S rDNA and in the 16S–23S internal transcribed spacer was investigated. Similar strains, previously reported in the literature, had been assigned either to the species *M. intracellulare* or to the group of MAC intermediates. However, several phenotypical and epidemiological characteristics seem to distinguish these strains from all other MAC organisms. The unique mycolic acid pattern obtained by HPLC is striking as it is characterized by two clusters of peaks, instead of the three presented by all other MAC organisms. All of the strains have been isolated from humans and all but one came from the respiratory tract of elderly people. The clinical significance of these strains, ascertained for seven patients, seems to suggest an unusually high virulence. The characteristics of all the strains reported in the literature, genotypically identical to the ones described here, seem to confirm our data, without reports of isolations from animals or the environment or, among humans, from AIDS patients. Therefore, an elevation of the MAC variant was proposed and characterized here, with the name *Mycobacterium chimaera* sp. nov.; this increases the number of species included in the *M. avium* complex. The type strain is FI-01069T (=CIP 107892T=DSM 44623T).—Authors’ Abstract


*Mycobacterium heckeshornense* is a rare isolate in clinical specimens. We performed simultaneous 16S rRNA sequence analysis of a mycobacterium culture and a histopathology specimen to determine the relevance of *M. heckeshornense* infection in an immunocompetent patient initially presenting with pneumothorax.—Authors’ Abstract


Tuberculosis continues to be a major killer disease, despite an all-out effort launched against it in the postgenomic era. We describe here the population structure of *Mycobacterium tuberculosis* strains, as revealed by a chromosome-wide scan of fluorescent amplified fragment length polymorphisms (FAFLPs), for more than 1100 independent isolates from 11 different countries. The bacterial strains were genotyped based on a total of 136 ± 1 different FAFLP markers at the genome sequence interface, with details on IS6110 profiles, drug resistance status, clinicopathological observations, and host status integrated into the analysis process. The strains were found to cluster with possible geographic affinities, including the parameters of host species type, IS6110 profile, and drug susceptibility status. Of the five most commonly amplified fragment sets (or amplitypes), type A predominated in strains of mixed origin, deposited in The Netherlands; type B was exclusively observed for Indian isolates; type C was found mainly in strains from Peru and Australia; and types D and E predominated in European strains from France and Italy. The amplitypes were independent of certain large sequence polymorphisms representing two important deletions, TbD1 and Rd9. It appears that *M. tuberculosis* has a high genomic diversity with a possible geographic evolution. This may have occurred due to specific genomic deletions and synonymous substitutions selected rigorously against host defenses and environmental stresses on an evolutionary timescale. The genotypic data reported here are additionally significant for genotype-phenotype correlations and for determining whether pathogen diversity is a reflection of the host population diversity. — Authors’ Abstract


This special microarray issue of Tuberculosis recognises the important contributions of *M. tuberculosis* whole genome DNA microarrays to tuberculosis research by bringing together a range of papers that address *M. tuberculosis* physiology, host-pathogen interactions, mechanisms of drug action, *in vitro* and *in vivo* gene expression, host responses, comparative genomics and functional analysis of particular genes. A number of complete datasets of *M. tuberculosis* mRNA expression levels are provided to facilitate multiple cross-condition comparison. Microarrays represent one of the new functional genomics technologies exploiting genome sequence information that will bring us closer to realising the scientific and moral imperatives of better vaccines, diagnostics and new drugs for the control of tuberculosis throughout the world. — Author’s Abstract


We have analyzed, using complementary molecular methods, the diversity of 43 strains of “*Mycobacterium canettii*” originating from the Republic of Djibouti, on the Horn of Africa, from 1998 to 2003. Genotyping by multiple-locus variable-number tandem repeat analysis shows that all the strains belong to a single but very distant group when compared to strains of the *Mycobacterium tuberculosis* complex (MTBC). Thirty-one strains cluster into one large group with little variability and five strains form another group, whereas the other seven are more diverged. In total, 14 genotypes are observed. The DR locus analysis reveals additional variability, some strains being devoid of a direct repeat locus and others having unique spacers. The hsp65 gene polymorphism was investigated by restriction enzyme analysis and sequencing of PCR amplicons. Four new single nucleotide polymorphisms were discovered. One strain was characterized by three nucleotide changes in
441 bp, creating new restriction enzyme polymorphisms. As no sequence variability was found for hsp65 in the whole MTBC, and as a single point mutation separates *M. tuberculosis* from the closest “*M. canetti*” strains, this diversity within “*M. canetti*” subspecies strongly suggests that it is the most probable source species of the MTBC rather than just another branch of the MTBC.—Authors’ Abstract
ACKNOWLEDGMENT

The Board of Directors of the INTERNATIONAL JOURNAL OF LEPROSY gratefully acknowledges the financial assistance from special grantors and sustaining members which, with the special donations of certain members, has made possible the continuation of publication of the JOURNAL directly by the International Leprosy Association. Without this assistance the official organ of the ILA, so essential to leprosy workers everywhere, could not be published.

SPECIAL GRANTORS

*Aide aux Lepreux Emmaus-Suisse, 9 Spitalgasse, CH-3011 Berne, Switzerland.

*American Leprosy Missions, One ALM Way, Greenville, South Carolina 29601, U.S.A.

Damien-Dutton Society, 616 Bedford Avenue, Bellmore, New York 11710, U.S.A.

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*Italian Association Amici di Raoul Follereau (AIFO), Italiana Raoul Follereau, Via Borselli, 4-6 40135 Bologna, Italy.

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