In this issue, the JOURNAL initiates a new feature, “Images from the History of Leprosy.” Of all the major maladies of mankind, few have a history as extraordinary and as well-documented as leprosy. We believe that this JOURNAL provides an appropriate place in which to collect a series of images portraying the depth and richness of this history, and issue an open invitation to members of the International Leprosy Association, as well as to other physicians, friends, and institutions who may have in their possession valuable images from this multifaceted history.

Of the greatest interest for this feature are photographs, sketches, or other images that illustrate events, discoveries, institutions, and ideas that have played a significant role in the history of leprosy. In order to avoid having this feature become only a gallery of physicians and scientists who have worked on leprosy, we do not wish to encourage submission of photographs of these individuals, believing that their contributions are more appropriately recognized in other ways.

Two major criteria will be applied for the selection of images for this feature: first, that the subject is important in the history of leprosy, and second, that the image itself is of high quality. Those who submit images for consideration are asked to provide as much documentation as possible concerning the subject of the image, as well as documentation about the image itself (including the source or artist, medium, and dates of creation or publication if known). If you would like to contribute an image for consideration, please communicate first with the JOURNAL office (ijl@lsu.edu) to describe the nature of the image. Please do not send originals; we will provide contributors with information about the preferred methods of electronic (or other) reproduction for publication.

This series begins with the haunting portrait of a 14-year-old girl published in the landmark atlas of Daniellsen and Boeck in 1847. The artist has carefully recorded the clinical details of the macular lesions on her cheeks, but has also captured in her eyes the bewildered look of sadness and apprehension that is familiar to generations of physicians who have had the task of advising their patients of this diagnosis.
Reproduced here is Planche IX from the original *Atlas Colorié de Spedalskhed [Atlas of Leprosy]* by D. C. Daniellsen and C. W. Boeck. This Atlas is a landmark in the medical history of leprosy, as it represents the beginning of the modern understanding and classification of this disease.

The Atlas was printed by Trykt i Prahls Lithographs in Bergen, Norway, in 1847. The image here is reproduced electronically from an original chromolithograph made from a painting by J. L. Losting. The lithograph measures 49.5 cm × 33.0 cm.

This image and documentation were contributed by the Section of Rare Books—Library Luiza Keffer—Instituto Lauro de Souza Lima, Bauru, Brazil.

This image may be viewed in color in the electronic edition of the Journal. Please visit our web-site at leprosy-ila.org, and click on the Journal icon.
Epidemiological Characteristics of Leprosy Reactions: 
15 Years Experience from North India

Bhushan Kumar, Sunil Dogra, and Inderjeet Kaur

ABSTRACT

A retrospective analysis of patient’s leprosy clinic records at PGIMER, Chandigarh, India for the period 1983 to 1998 was undertaken to study the frequency, time of onset, and risk factors for leprosy reactions. Of the 2600 cases analyzed, 1494 were multibacillary and 1106 had paucibacillary disease. Presentation with reaction was common with 30.9% of our patients having reactions at the time of first visit. The incidence of reversal reaction (RR) was highest during 6 to 12 months after starting multi-drug therapy (MDT), thereafter declining gradually. Late RR occurred in 9.5% of all cases and was noted up to 7 years after treatment. Female gender, widespread disease, and multibacillary disease were identified as risk factors for RR. Erythema nodosum leprosum (ENL) reactions were noted to occur mostly during second or third year after starting MDT. Of the total number of patients who experienced ENL, 64.3% had recurrent episodes which continued for up to 8 years after the start of treatment. Lepromatous leprosy, female gender, and high Bacterial Index (≥3) were recognized as risk factors for developing ENL. Occurrence of recurrent and late reactions, even though of mild severity, highlights the importance of recognizing and treating them promptly to prevent or reduce morbidity, complications, and further deterioration in the disability status. Although it is hoped that leprosy will have been eliminated at all levels by 2005, the recognition and management of these reactions will continue to be the most essential/significant task in the post elimination era.
With the success of multi-drug therapy (MDT) in the treatment of leprosy, attention has focused on the problem of leprosy reactions, which are now the most significant issue in the management of individual patients. Despite a large burden of leprosy cases in India, very limited data has been published on the epidemiology of reactions from this part of the world. The available information about the epidemiology of leprosy reactions is incomplete and scanty, despite a growing amount of literature on its treatment.

Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, is a tertiary care institute in Northern India, which is a low endemic area for leprosy. Many patients with leprosy are self-referred and some are referred by a doctor or clinic, citing the better quality of care available at our center. In addition to the population of this region, the institute also caters to a large migrant population from various states of the country where leprosy is endemic. In the leprosy clinic at the institute, a good record keeping system combined with regular evaluation of patients has generated a very large and useful database for retrospective analysis of reactions in leprosy. Systematic analysis of these records was carried out to determine the incidence of leprosy reactions in our patients, and to identify risk factors if any. In this paper, we discuss the incidence and risk factors for leprosy reactions over the last 15 years.

MATERIALS AND METHODS

A total of 2867 new, previously untreated patients enrolled in the leprosy clinic at PGIMER during the period from 1983 to 1998 were included in the study. Patients with pure neuritic leprosy were excluded from this analysis. Until 1987 all patients with Bacterial Index (BI) ≥2 were classified...
as multibacillary (MB), and <2 as paucibacillary (PB). From 1988 to 1998, all patients having positive slit skin smear were assigned to the MB group. Until 1994, all MB cases were treated with WHO MDT MB regimen for a minimum period of 2 yrs or until skin smear negativity, whichever occurred last. From 1994 to 1998, they were treated with fixed duration (24 months) MDT MB regimen.

After release from treatment (RFT), patients were seen at intervals of 3 to 6 months for the initial 2 years and later once or twice a year. Apart from this, patients were instructed on features of reactions/relapse, and told to attend the clinic immediately if they ever experience such symptoms or any other problems. A detailed clinical examination was done on each visit and skin smears are taken at least once a year. Details about time of onset and clinical features of reactions are recorded in the patient’s record file.

Reversal reaction (RR) involving skin was diagnosed if the patient had redness and swelling of (already existing) lesions, or the appearance of a few new lesions close to the existing lesions or at distant sites, with or without tenderness of lesions. Neuritis as a part of reaction was defined as spontaneous pain (shooting, tingling, or burning), or tenderness of the nerves with nerve function impairment (NFI). In most patients with reactions, features of concomitant skin and nerve involvement were present. Erythema nodosum leprosum (ENL) was diagnosed if a patient developed multiple, usually small, tender, evanescent nodules, with or without ulceration, which were usually associated with constitutional symptoms. Wherever indicated, reactional episodes were treated with oral prednisolone (40–60 mg/day) tapered to a stop over 12 weeks. Occurrence of a RR after six months of stopping MDT was labeled as late RR. Recurrent episodes of RR or ENL were defined as having a recurrence of symptoms of either type of reaction more than six weeks after the completion of treatment for reaction. Recurrence of a reactional episode earlier than this was considered to be possibly due to inadequate or abrupt stoppage of treatment. The exact definitions of the spectrum of the disease and duration of treatment with MDT were not uniform in all the patients due to changing definitions of PB/MB cases by the WHO; however, from a purely academic/research point of view we have also tried to analyze trends of reactional events at presentation, during MDT, and after release from treatment, according to Ridley Jopling classification (5) among subgroups of PB/MB cases. For validation of our supposition and better understanding, we have used certain terminologies like “disseminated disease” and “late ENL” because of the absence (or not very satisfactory) definitions about reactions. Patients were labelled as having “disseminated disease,” if there were ≥3 body areas involved, 6 or more skin lesions, or involvement of at least 2 peripheral nerve trunks. We have used the terminology “late ENL” for occurrence of ENL reaction 2 years after MDT completion.

The chi square test was used to analyze the difference between proportions and to identify trends. The difference between two unpaired sample means was tested using the student’s t-test. The significance of various risk factors for developing reactions was analyzed with Cox’s proportional hazards regression, and the results were expressed as rate ratios. Of the ratio, the 95% confidence interval is given.

RESULTS

Patients. Out of these 2867 patients, 62 patients were excluded, either because the diagnosis was changed or records were not complete. Another 205 patients were lost to follow-up or left the area after diagnosis. A total of 2600 patients with follow-up available up to 13 yrs (at least 3 yrs) were eligible for analysis. Average period of follow-up was 74 months (range 36 to 156 months). There were 1634 males (mean age 36 ± 3.2 yrs) and 966 females (mean age 39 ± 2.3 yrs). Out of them, 1494 (57.5%) had MB and 1106 (42.5%) had PB disease.

Incidence. The incidence of reversal reactions (RR) and erythema nodosum leprosum (ENL), and their distribution in PB/MB cases at the time of first presentation to the clinic is given in Table 1. The incidence of RR was 24.1% (627/2600 patients) and the figure for ENL in MB cases was 11.8% (177/1494 patients). Of the 627 patients with RR, 169 (27%) had evidence of reaction in skin lesions only and the re-
remaining 458 (73%) had involvement of both skin and nerves, whereas in patients having ENL, 86 (48.6%) had only cutaneous involvement and 91 (51.4%) had involvement of both skin and nerves.

In the total period of observation, 858 patients experienced RRs and 337/1494 MB cases had ENL either at the time of registration, during, or after release from treatment with cumulative incidence of 33% and 22.5%, respectively. Altogether, there were 1356 episodes of RR in 858 patients (1.6 reaction episodes/patient) and 885 episodes of ENL in 337 patients (2.6 reaction episodes/patient). Of these 337 MB cases, 203 were treated before 1994 and the rest received fixed duration (24 months) MDT (p <0.05).

**Time of onset.** Figures about the reactional episodes according to the period of time they occurred are given in Table 2. A great majority of RRs occurred during the first 6 months after starting MDT whereas, the episodes of ENL occurred at a higher frequency in the second or third year after starting MDT.

**Late reactions.** Late reversal reactions were seen in 4.2% (47/1106) of PB patients and 5.3% (79/1494 patients) with MB disease. The majority of late reactions in the multibacillary group were observed in patients with borderline lepromatous (BL) disease. The incidence of late RR was highest during the first 2 yrs after being released from treatment (RFT). Even though the decline in the incidence of RR began early after the initiation of therapy, and most reactions occurred during the first 2 yrs, a few continued to occur until 7 yrs after RFT.

Late ENL reactions were seen in only 3% (45/1494) of MB [BL and polar lepromatous (LL)] patients. Late ENL, though mild and usually not associated with significant constitutional symptoms, continued to occur for up to 8 yrs after completion of MDT. Of these patients, 15 were treated be-

### Table 1. Prevalence of reactions at the time of first presentation.

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. of cases</th>
<th>Reversal reaction</th>
<th>ENL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Skin only</td>
<td>Skin + nerves</td>
<td>Total</td>
</tr>
<tr>
<td>MB</td>
<td>1494</td>
<td>108</td>
<td>291</td>
<td>399 (26.7)</td>
</tr>
<tr>
<td>PB</td>
<td>1106</td>
<td>61</td>
<td>167</td>
<td>228 (20.6)</td>
</tr>
<tr>
<td>Total</td>
<td>2600</td>
<td>169</td>
<td>458</td>
<td>627 (24.1)</td>
</tr>
</tbody>
</table>

### Table 2. Time of onset of reactional episodes.

<table>
<thead>
<tr>
<th>Period</th>
<th>Paucibacillary</th>
<th>Multibacillary (N = 1494)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT (N = 1106)</td>
<td>BB (N = 82)</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Reversal reactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At registration</td>
<td>228</td>
<td>44.5</td>
</tr>
<tr>
<td>0–6 months</td>
<td>144</td>
<td>28.1</td>
</tr>
<tr>
<td>7–12 months</td>
<td>65</td>
<td>12.6</td>
</tr>
<tr>
<td>2nd year</td>
<td>46</td>
<td>9.0</td>
</tr>
<tr>
<td>≥3 years</td>
<td>29</td>
<td>5.7</td>
</tr>
<tr>
<td>Total</td>
<td>512</td>
<td>100</td>
</tr>
</tbody>
</table>

ENL Reactions

<table>
<thead>
<tr>
<th>Period</th>
<th>PAUCIBACILLARY</th>
<th>MULTIBACILLARY (N = 1494)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT (N = 1106)</td>
<td>BB (N = 82)</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>At registration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–12 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥3 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Period</th>
<th>PAUCIBACILLARY</th>
<th>MULTIBACILLARY (N = 1494)</th>
</tr>
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<tr>
<td></td>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Reversal reactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At registration</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
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<tr>
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</tr>
<tr>
<td>2nd year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥3 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
fore 1994 and the remaining 30 with fixed duration regimen (24 months) (p < 0.01). However, there was no statistically significant difference among the number of MB patients manifesting late RRs treated before 1994 (43/79) and those treated with fixed duration regimen (36/79) (p > 0.1).

**Recurrent reactions.** Of the 858 patients who manifested reversal reactions, 252 (29.4%) had ≥ 2 episodes and the remaining 606 (70.6%) had only a single episode of RR. Cumulative incidence of recurrent reversal reaction was 9.7% (252/2600) among all of the patients. The presence of a reversal reaction at the time of initial presentation or during the first year of treatment was more frequently associated with an increased risk of having another episode occurring later on.

Out of the total patients who experienced ENL, 217/337 (64.4%) experienced more than one episode. The median number of episodes was 4 and the time between the first and last episode averaged 34 months (range 5 to 96 months). A considerable number of patients (51/217, 23.5%) had more than ≥ 4 episodes of ENL over a period of observation varying from 3 years to 8 years, with no other identified risk factor except a higher BI (BI ≥ 3).

**Risk factors.** Female gender, multibacillary disease, and widespread disease (≥ 3 body areas involved, ≥ 6 skin lesions, or ≥ 2 peripheral nerve trunks involvement) were statistically significant risk factors for developing reversal reactions. The strongest association was observed between the extent of clinical disease and the risk of developing RR. Of the 858 patients manifesting RRs, 544 (63.4%) had ≥ 3 body areas involved and/or ≥ 6 skin lesions, and 523 (61%) had ≥ 2 nerve trunk involvement. Age was not found to be a significant risk factor for reversal reactions (Table 3).

Risk factors for developing ENL reactions are given in Table 4. Lepromatous leprosy, female gender and higher BI (≥ 3) were significant risk factors, whereas age was not.

**DISCUSSION**

**Incidence.** Much is known about the epidemiology of reactions, but their incidence in the period after MDT is less well documented because of lack of long term follow-up (6). Various estimates of the frequency of reversal reactions have been given by several authors (1-6, 17). Published reports indicate that the frequency of RR at the time of diagnosis varies between 2.6% and 6.4% (6) though a much higher figure of 28% was reported in a hospital-based study from Nepal (17). In our study, this figure was 24.1%. Other studies have reported relatively lower total figures of 9.1% from Hyderabad, India (10) and 16.5% from Ethiopia (14), probably reflecting a variable proportion of PB/MB cases and the use of mixed case definitions used. Figures for the percentage of patients manifesting RRs at any time vary from

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Patient group PB/MB/Both</th>
<th>No.</th>
<th>Rate ratio* (95%CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>314/1106</td>
<td></td>
<td>0.69 (0.58–0.81)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>MB</td>
<td>544/1494</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Both</td>
<td>120/357</td>
<td>1.0 (0.81–1.3)</td>
<td>p &gt; 0.1</td>
</tr>
<tr>
<td>≤ 20</td>
<td>Both</td>
<td>738/2243</td>
<td>0.80 (0.68–0.95)</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>Both</td>
<td>510/1634</td>
<td>2.5 (2.1–2.9)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Sex</td>
<td>Both</td>
<td>348/966</td>
<td>2.4 (2.0–2.8)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Male</td>
<td>Both</td>
<td>544/858</td>
<td>2.8 (2.2–3.1)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Female</td>
<td>Both</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extent of clinical disease</td>
<td>Both</td>
<td>523/858</td>
<td>2.4 (2.0–2.8)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>≥ 6 skin lesions</td>
<td>Both</td>
<td>544/858</td>
<td>2.8 (2.2–3.1)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>≥ 2 nerves involved</td>
<td>Both</td>
<td>523/858</td>
<td>2.4 (2.0–2.8)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>≥ 3 Body areas involved</td>
<td>Both</td>
<td>544/858</td>
<td>2.8 (2.2–3.1)</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

*Rate ratio adjusted for the influence of age and sex.
3.5% among PB cases in Malawi (1) to 47.5% among MB cases in Zaire (6). Due to the use of widely different case definitions, it is difficult to compare the frequencies of RR in PB and MB patient groups in studies published from different centers. Other factors contributing to such variation could be the time between diagnosis and beginning MDT, duration of MDT, duration of steroid regimen, and quality of the local leprosy control program. Overall 33% of all patients in our study developed RR at some time during treatment and follow-up, including those with a reaction at presentation.

ENL reactions were reported to occur in more than 50% of lepromatous leprosy (LL) cases and in about 25% of borderline lepromatous (BL) cases in the pre-MDT era (7). The incidence of ENL reactions appears to have fallen with the introduction of MDT, possibly due to the combined bactericidal effect of rifampicin and the anti-inflammatory effect of clofazimine in suppressing ENL (8). A hospital-based study from Nepal reported a high frequency of ENL reactions (28.6%) in LL, but only 7.5% in BL cases (9). In the present study, 47.4% of LL cases and 10.5% of BL cases manifested ENL reactions. The lower incidence of ENL among patients treated before 1994 could be because of the persisting anti-inflammatory effect of clofazimine till bacterial clearance was achieved. Field studies have reported a rather lower incidence of ENL such as 12% in LL and 3.6% in BL from Ethiopia (10), and 2.1% of all MB patients from Bangladesh (10). The varied frequency of ENL in reported studies could be due to patients in the field screened for reactions vs. patients reporting to the hospitals for reactions. Most of our patients were self-reporting having reactions severe enough to force them to seek treatment from a hospital rather than field-based clinics. This could be one reason for relatively higher figures for ENL in our center.

In reactions (RR or ENL) involvement of the skin and nerves occurred either singly or together. Of the total number of reactions at the time of presentation, 31.7% had only cutaneous involvement, whereas 68.3% had involvement of both skin and nerves. In a retrospective analysis of reversal reactions in a study from Hyderabad, India, 43.1% had only skin lesions, 31.8% had only neuritis, and 22.7% had both skin lesions and neuritis (6). Such observations emphasize that neuritis can occur along with inflammatory skin lesions or independently. Therefore, even very mild symptoms suggestive of neuritis should be taken seriously and nerves should be palpated on each visit to detect early signs of nerve inflammation, regardless of presence or absence of reaction involving skin.

**Time of onset of reactions.** Significantly, 30.9% of our patients presented to us because of reactions, in spite of having symptoms suggestive of leprosy for months or years. In a hospital-based study from Hyderabad, India, Lockwood, et al. (6) noted reactions in a strikingly high percentage of their patients (41.3%) at the time of presentation. It is obvious that many patients seek treatment only when they get frightened by the sudden development of such lesions particularly over face, or painful symptoms of neuritis due to reaction.

Although it is known that the reversal reactions occur most frequently within 6 to 12

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**Table 4. Risk factors for development of ENL reactions.**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Variables</th>
<th>No.</th>
<th>Rate ratio*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease group</td>
<td>BL</td>
<td>95/902</td>
<td>.12 (0.09–0.17)</td>
<td>p &lt;0.01</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>242/510</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>&lt;20</td>
<td>34/238</td>
<td>1.1 (0.75–1.6)</td>
<td>p &gt;0.1</td>
</tr>
<tr>
<td></td>
<td>&gt;20</td>
<td>303/2322</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>192/1634</td>
<td>.75 (0.59–0.95)</td>
<td>p &lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>145/966</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI</td>
<td>&lt;3</td>
<td>120/2109</td>
<td>07 (0.05–0.09)</td>
<td>p &lt;0.01</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>217/491</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Rate ratio adjusted for the influence of age, sex and bacteriological index.
months after starting treatment (4, 11), most previous studies did not report long term follow-up. In the AMFES data (Ethiopia), RR were reported to occur as late as 5 yrs after the start of treatment in both PB and MB patients (12). Our study also supports the fact that though the incidence of RR was found to decline gradually, reactions continued to occur for 7 years, though in very few patients. However, in India the longest interval reported between treatment and reaction is 6.5 years (6).

The majority of ENL reactions after starting MDT occurred in the second or third year and the recurrence of attacks was mostly noted in the same period, though the reactions continued to occur up to 8 yrs after RFT.

**Late reactions.** The percentage of patients developing late RR in both PB and MB groups was 4.2% and 5.3%, respectively. In a study from Thailand, 2.7% of PB patients and 9% of MB patients developed a late reversal reaction (15). In Malawi, 3.5% of PB patients developed late reactions during the first 4 yrs of follow-up (7). In a cohort of MB patients (treated with MDT for 2 yrs or longer until split-skin smear tested negative) from Karigiri, India, only 1.1% experienced reversal reactions during almost 10 yrs of surveillance (18). Late RR is known to occur mostly within the first 3 to 4 yrs after RFT (1, 15) as was also observed by us. In the absence of a clear definition for late RR and different MDT regimens used in various studies, an exact comparison of the frequency of late RRs is not possible.

Late ENL reactions were recorded in 3% of our MB cases. The majority of these episodes consisted of a few ENL lesions, which were mostly associated with only mild cutaneous and constitutional symptoms.

**Recurrent reactions.** Recurrent episodes of reversal reactions are an important clinical phenomenon, which may result in continuing nerve damage and add on to the degree and number of impairments. In our study, 9.7% patients developed recurrent episodes of reversal reaction. Almost half of these episodes occurred within 3 months of stopping the course of prednisolone that had been administered for the previous reaction. Strikingly, in a hospital-based study from Hyderabad, India, 33% of patients with RRs had recurrent episodes (9). The immune suppression in some of these patients may have been for of too short a duration, or hospitals may be more likely to get the severely affected problem patients, which require still longer treatment with steroids or some other adjuvant therapy. Possibly, Naafs, et al. may be right when they suggest that immunosuppressive treatment should continue throughout the period when the antigen load is sufficient to trigger the cell mediated immune response (10, 11).

Of all patients who manifested ENL, 64.3% had recurrent episodes. In the AMFES cohort (Ethiopia), 63% of all cases with ENL had more than one episode, and 31% of all ENL cases manifested 5 or more episodes over a period of more than 2 yrs. In general, recurrence in episodes of reactions is more common in ENL than in RR, and approximately one-third of patients with ENL reactions go on to have recurrent episodes (14, 16). Patients must be warned of this possibility and be educated to return for follow-up, and clinicians should also be aware to diagnose even very late reactions in post-elimination era. Though it could be in variance to other proposed definitions (Transactions of 16th International Leprosy Congress), we suggest that a patient could be labeled as having “chronic ENL” if he needs continued antireaction treatment for a period of 6 months or more.

**Risk factors.** Risk factors for RR identified to be significant in this study were female gender, and disseminated disease (extent of clinical disease measured by involvement of a number of body areas, nerves, and skin lesions) at the time of diagnosis. PB patients had less risk of developing reversal reactions than MB patients. Patients with three or more body areas involved or having ≥6 skin lesions had about twice the risk of developing RR than those with limited disease (63.4% vs. 36.6%). Similar observations (>10 skin lesions) have been made by Van Brakel, et al. (17), which suggested that this can be of considerable importance for control programs, to identify patients at risk of developing reactions. They argued that “body area” may just be a proxy indicator for the bacteriological index or multibacillary end of the leprosy spectrum. Like our observation,
they also noted similar association within the borderline tuberculoid (BT) patient subgroup, indicating that body area count is useful indicator of the risk of developing RR. Pregnancy and lactation are reported to be risk factors for RR and ENL (6,8), but the association has not been quantified and remains unclear. Leprosy lesions over the face have also been observed to develop RR more frequently (9). However, the relation between pregnancy or lactation and leprosy reactions, the significance of a patch over the face as a risk factor for developing reversal reactions, could not be statistically analyzed in the present study due to lack of complete information in the records.

For ENL, the risk factors identified in our study were lepromatous leprosy, female gender, and higher bacteriological index (≥3). For recurrent ENL, a number of risk factors like age, sex, spectrum of disease were analyzed, but only high BI (≥3) was found to be statistically significant. In AMFES cohort (Ethopia), no specific risk factor for recurrent ENL could be identified except age, between 20 and 45 yrs (14). In our study, though the majority of cases were in this age range, correlation with any age group could not be confirmed. ENL reactions are reported to occur throughout pregnancy and lactation, and may be severe and recurrent; however, because of incomplete information as stated above, this relationship could not be analyzed in our cohort.

In conclusion, this is the latest series of patients with long term follow-up, delineating the epidemiology of reactions in leprosy from India. RR and ENL are common complications in leprosy patients in India. Female gender, multibacillary leprosy, and extensive disease were found to be major risk factors for occurrence of RRs. The majority of RRs occurred within 12 months of starting MDT, and then the incidence declined gradually but the reactions continued to occur until 7 yrs after RFT. For ENL, lepromatous leprosy, female gender and higher bacteriological index (≥3). Significant risk factors were approximately one-third of patients with ENL reactions go on to manifest recurrent episodes spread out over a period of more than 2 yrs requiring specialized expertise to manage them. Though leprosy is expected to be eliminated from all nations by 2005, even those patients who have successfully completed their treatment will continue to manifest with late or recurrent reactions in settings of poorly available expertise or services to manage these episodes.

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Neuropathic Pain in Leprosy Patients


ABSTRACT

The introduction of multidrug therapy by the World Health Organization has dramatically reduced the world prevalence of leprosy but the disease is still a public health problem in many countries, with a world prevalence of almost 600,000 cases in 2001. Damage to peripheral nerves is a key component of leprosy and the sensory and motor loss that follows is the basis for many of the classical features of this disease, such as skin wounds, cracks, plantar ulcers, clawed hands, drop foot, and incomplete closure of the eyelids. One of the most remarkable aspects of leprosy to lay persons and health care workers alike is that patients are reputed to feel no pain. However, neuropathic pain is arising as a major problem among leprosy patients. It can be nociceptive due to tissue inflammation, which mostly occurs during episodes of immune activation or neuropathic due to damage or dysfunction of the nervous system. This study, conducted among 358 leprosy patients, reveals a considerable prevalence of neuropathic pain and presents evidence that this common problem should be a high priority of those in charge of leprosy control programs.

RÉSUMÉ

L’introduction de la poly-chimiothérapie par l’Organisation Mondiale de la Santé a diminué de façon drastique la prévalence mondiale de la lèpre mais la maladie est encore un problème de santé publique dans plusieurs pays, avec une prévalence mondiale de presque 600 000 cas en 2001. L’atteinte des nerfs est une composante clef de la lèpre et les pertes sensorielles et motrices qui s’ensuivent forment la base des caractéres classiques de cette maladie, comme les blessures cutanées, les fissures, les ulcères de la plante des pieds, les mains en crochets, les pieds tombants et la fermeture incomplète des paupières. Un des aspects les plus remarquables de la lèpre pour le grand public comme pour le prestataire de soins de santé est que les patients ont la réputation de ne pas ressentir de douleur. Cependant, les douleurs neurogènes sont en train d’émerger comme un problème majeur parmi les patients hanséniens. Elle peut être nociceptive, due à l’inflammation qui apparaît principalement durant les épisodes d’activation immunologique, ou bien neurogène, causée par une atteinte ou une dysfonction du système nerveux. Cette étude, menée chez 358 patients lépreux, révèle une prévalence considérable de douleurs neurogènes et présente des arguments afin que les responsables de santé publique et de contrôle de la lèpre considèrent ce problème comme une priorité.

RESUMEN

No obstante que la introducción de la poliquimioterapia por la Organización Mundial de la Salud ha reducido dramaticamente la prevalencia de la lepra a nivel mundial, la enfermedad es todavía un problema de salud pública en muchos países, con una prevalencia de casi 600,000 casos en 2001. El daño a los nervios periféricos es un componente crítico de la lepra y la pérdida sensorial y motora que le siguen es la base de muchas de las características clásicas de la enfermedad que incluyen heridas en la piel, cuarteaduras, úlceras plantares, manos en garra, pie caído y cierre incompleto de los párpados. Uno de los aspectos más marcables de la lepra es la creencia general de que los pacientes no sienten dolor. Sin embargo, el dolor neuropático se está manifestando como un problema cada vez mayor entre los pacientes con lepra. El dolor puede ser enmascarado por la inflamación del tejido que ocurre principalmente durante los episodios de activación inmune o neuropática asoci-
Since the introduction of an effective treatment based upon a multi-drug therapy (MDT) of dapsone, clofazimine, and rifampicin as recommended by the World Health Organization (WHO), the prevalence of leprosy has dramatically decreased (13). However, the disease is still a public health problem in many countries with an estimated global prevalence of near 600,000 cases as for the year 2003 (14).

Damage to peripheral nerves is a key component of leprosy and, together with typical skin lesions, accounts for the major traditional clinical features of the disease. Little is known about the mechanism by which the mycobacteria infect Schwann cells, but recently some evidence has emerged. A glycoprotein (α-dystroglican) that binds to the surface of *Mycobacterium leprae* also binds to a molecule on the surface of the Schwann cell surface and provides a potential mechanism for internalization of the bacilli by Schwann cells (1, 9).

The sensory and motor loss that follows nerve damage in leprosy is the basis for many of the classical features of the disease such as skin wounds, cracks, plantar ulcers, clawed hands, drop foot, and lagophtalmos. Sensory damage includes an early loss of pain and temperature perception followed by compromise of tactile and pressure senses. The distribution and onset of nerve damage can vary according to the type of leprosy, being more disseminated and gradual in the lepromatous cases, or localized and acute in tuberculoid and borderline cases. The indeterminate type is an initial presentation of the disease in which major nerve damage has not yet developed.

One of the most remarkable aspects of leprosy to lay persons and health care workers alike is that patients are reputed to feel no pain. This widespread impression is rapidly changing as many patients who have completed their WHO MDT are now reporting complaints of stimulus-independent ongoing pain, and seeking relief. Indeed, pain in leprosy can be nociceptive due to tissue inflammation, which mostly occurs during episodes of immune activation [“reversal reaction” (RR) and “erythema nodosum leprosum” (ENL)] or neuropathic due to damage or dysfunction of the nervous system. Nociceptive pain is due to activation of peripheral nociceptors on A-delta and C-fibers, secondary to nerve tissue injury during ENL or RR. There is a release of bradykinin, serotonin, substance P, histamine and prostaglandin, which facilitate the transmission of pain impulses from the periphery to the spinal cord.

The complex and stigmatizing burden of being diagnosed with leprosy may compel patients to focus solely upon curing the disease, which is actually accomplished with the WHO drug regimen, and to accept their symptoms as an inevitable concomitant or residual of the disease. However, the number of cases with pain problems seemed to us to be substantial (4). The aims of this study, conducted in a country where leprosy is endemic, were to estimate the prevalence of pain in patients with leprosy and to determine the main characteristics of their pain.

**MATERIALS AND METHODS**

The study was conducted at the Instituto Lauro de Souza Lima, Bauru, Brazil, a national referral center for leprosy patients. Brazil has a prevalence of 77.676 cases per 100,000, and an important detection rate of 24.1/100,000 (41.070 new cases in 2000), which makes it the second largest endemic country for leprosy in the world after India (14).

The study included 358 patients with leprosy who presented to the Dermatological clinic of Instituto Lauro de Souza Lima from October 1, 2001 to March 31, 2002. Among them, 215 were male (60.1%) and 143 were female (39.9%). The mean age was 54.8 yrs (S.D. = 16 yrs), with a range of 11 to 87 yrs.

The mean time from initial diagnosis was 18.3 yrs (range, 10 months to 68 yrs, S.D. = 18.5 yrs), and 178 (49.7%) patients were diagnosed over 10 years earlier. According to
the Madrid Classification (8), 207 patients (57.8%) were lepromatous, 92 (25.7%) borderline, 54 (15.1%) tuberculoid, and only 5 (1.4%) were indeterminate. All cases were receiving treatment except for 283 (79.1%) patients who had concluded their standard course by the time of the study. Treatment regimens included the WHO MDT, dapsone plus rifampicin, or dapsone as monotherapy in some previously treated cases.

Two hundred and one (56.1%) of the patients reported past or current moderate to severe chronic neuropathic pain that interfered with activities of daily living or disturbed sleep. None of these cases revealed signs or symptoms of RR or ENL as assessed by an experienced clinician. These cases underwent clinical neurological examination by trained health workers, including detailed anamnesis assessment focusing on the occurrence of pain, its localization, duration, pattern of symptoms onset, quality, and quantity. Localization of pain refers to the anatomical distribution and trunk of the most relevant affected peripheral nerve. Duration of pain was classified as less or greater than 6 months. The pattern of symptom onset was categorized as abrupt, insidious, or in repetitive bursts. Assessment of quality of pain was based upon the Brazilian Portuguese version of the McGill Pain Questionnaire (12). In addition, we inquired whether present pain was experienced as superficial, deep, or mixed. Pain intensity was verbally rated by patients as mild, moderate, or severe and was also rated on a graphic scale (empty to full water glass).

RESULTS

Using the day of interview and examination as the reference point, 148 (73.6%) patients reported episodes of pain only in the past and 53 (26.4%) had complaints at present. In the 148 patients with past pain only, leprosy had been diagnosed less than 10 years earlier in 59 cases (39.8%) and more than 10 years earlier in 89 cases (60.2%). In those with present pain, only 14 (26.4%) cases had been diagnosed over 10 years earlier. Pain had been present for 6 months or less in 55 cases (27.4%), whereas 141 (70.1%) reported pain for longer than 6 months. Only 5 patients (2.5%) could not estimate the duration of their pain. The nerve most often affected by pain was the ulnar (59.2%), followed by the tibial (30.3%), fibular (18.9%), median (4.5%), radial (2.0%), and trigeminal (1.5%). These percentages sum to greater than 100 because patients were free to indicate pain in the distribution of more than one nerve. Glove (22.4%) and stocking (24.9%) distribution of pain were also quite common (Fig. 1). The onset of episodes was reported as abrupt by 39 patients (19.4%), as insidious by 73 patients (36.3%), and as recurrent bursts by 89 (44.3%) patients. The assessment of quality of pain can be seen in Fig. 2.

In the 53 patients with pain present at the time of interview, the most affected anatomical layer was deep in 30 (56.6%) patients, superficial in 8 (15.1%) and mixed in 15 (28.3%). In these patients, pain was constant in 34 (64.2%) and episodic in 19 (35.8%). Verbal ratings of present pain were severe in 29 (54.7%), moderate in 17 (32.1%), and mild in only 7 patients (13.2%). On the graphic scale, 22 (41.5%) patients rated their pain as severe, 21 (39.6%) as moderate, and 10 (18.9%) as mild. These pain characteristics are summarized in The Table.

DISCUSSION

Lack of sensation is a paradigm of leprosy, and the diagnosis of this chronic infectious disease is assured by the presence of skin lesions (usually patchy) with marked sensory loss as assessed by Semmes-Weinstein monofilaments or a ballpoint pen tip. Abnormalities include loss of touch, temperature, and pressure sensation. Although clinical consensus regards leprosy as painless, in reality nerve pain in leprosy is often present during neuritis, a feature that accompanies acute leprosy reactions. These reactive episodes include entrapment of the nerve in selected sites (most often the ulnar canal at the elbow) due to edema from acute and severe inflammation of the nerve. In such a situation, activation of the nervi nervorum may be the main contributor to pain. Another possibility is that the acute neural inflammation can excite and sensitize nociceptors. In some cases, there is severe destruction of nerve fibers (7) and the partial regeneration that follows may produce discharges, diminution of stimulus thresholds, and exaggerated responses of nociceptors.
This study reveals that neuropathic pain not directly associated with an acute reactive episode may be present in a considerable proportion of patients with leprosy. In fact, out of 358 patients presenting to the outpatient dermatological clinic for other reasons, 56.1% reported prior or current episodes of neuropathic pain. Most of these patients reported that the intensity was severe and sufficient to interfere with activities of daily life or sleep.

According to the literature (3, 5), the most common nerve affected in leprosy is the ulnar nerve, and we confirmed the frequency of this as a painful site. However, a glove and stocking distribution of pain was also frequently reported by our patients. Although involvement of nerve trunks in leprosy is common, the superficial branches and their rami may be also compromised, particularly in lepromatous and borderline cases in which dissemination of the disease is a characteristic feature.

It is important to note that, among patients with present pain, 40 patients (75.4%) had completed antimicrobial treatment and, according to the present policy of leprosy control, are discharged from further follow-up. Such patients receive little further care. Therefore, a significant number of patients did not have ongoing access to care and could not seek assistance for relief of neuropathic pain and improvement of quality of life. In addition, 130 patients (87.8%) with past pain had already completed treatment by the time their pain occurred. Thus, successful completion of antimicrobial treatment does not appear to prevent occur-

### The Table: Some characteristics of neuropathic pain among 201 cases of leprosy with past (148) or present (53) pain.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Past pain*</th>
<th>%</th>
<th>Past pain*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical form</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepromatous</td>
<td>94</td>
<td>63</td>
<td>26</td>
<td>49</td>
</tr>
<tr>
<td>Borderline</td>
<td>29</td>
<td>20</td>
<td>20</td>
<td>38</td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>24</td>
<td>16</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Indeterminate</td>
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<td>0.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Onset</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Abrupt</td>
<td>30</td>
<td>20</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Insidious</td>
<td>47</td>
<td>32</td>
<td>26</td>
<td>49</td>
</tr>
<tr>
<td>Bursts</td>
<td>71</td>
<td>48</td>
<td>18</td>
<td>34</td>
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<tr>
<td>Duration in months</td>
<td></td>
<td></td>
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<tr>
<td>&lt;6 months</td>
<td>49</td>
<td>33</td>
<td>6</td>
<td>11</td>
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<tr>
<td>&gt;6 months</td>
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<td>89</td>
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<tr>
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<td>3</td>
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<tr>
<td>Time of leprosy diagnosis</td>
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<tr>
<td>&lt;10 years</td>
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<td>74</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>89</td>
<td>60</td>
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<td>Treatment completion</td>
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<tr>
<td>Yes</td>
<td>130</td>
<td>88</td>
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<tr>
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<td>18</td>
<td>12</td>
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<tr>
<td>Pain intensity</td>
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</tr>
<tr>
<td>Mild</td>
<td>—</td>
<td>10</td>
<td>19</td>
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<tr>
<td>Moderate</td>
<td>—</td>
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<tr>
<td>Severe</td>
<td>—</td>
<td>22</td>
<td>41</td>
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<tr>
<td>Verbal rating of pain intensity</td>
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<tr>
<td>Mild</td>
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<tr>
<td>Moderate</td>
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<tr>
<td>Severe</td>
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<td>29</td>
<td>55</td>
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<tr>
<td>Time of worst pain</td>
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</tr>
<tr>
<td>Morning</td>
<td>—</td>
<td>7</td>
<td>13</td>
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<td>Afternoon</td>
<td>—</td>
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<tr>
<td>Deep</td>
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<td>57</td>
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<tr>
<td>Mixed</td>
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<td>15</td>
<td>28</td>
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<tr>
<td>Evolution</td>
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<tr>
<td>Worsening</td>
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<td>20</td>
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<td>Remitting</td>
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<td>Character</td>
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<tr>
<td>Constant</td>
<td>—</td>
<td>34</td>
<td>64</td>
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</table>

*Number of patients
rence of neuropathic pain. As a matter of fact, the teams caring for patients with leprosy are not generally aware of the problem of neuropathic pain. Furthermore, when considering the indication for classical drugs for both RR and ENL, there appears to be some misunderstanding as to what is responsible for the pain component. Sometimes, it appears that health personnel believe that thalidomide or steroids can act as analgesics, which is not true. Of course, their action on reducing edema and attenuating immunological aspects of reaction can lead to a reduction in pain. Conversely, in pure neuropathic pain there is no room for such drugs. Therefore, it is of utmost importance that control of neuropathic pain in leprosy be included as an issue to be dealt with by leprosy control program managers. Control and treatment of neuropathic pain in leprosy has not yet been well studied. However, the experience with the treatment of such pain in other conditions has stimulated the use of some standard drugs in cases of leprosy (6, 7, 10, 11). In this regard, some good results have been reported while using tricyclic antidepressant drugs such as amitriptyline and imipramine. Anticonvulsant agents can also be used, such as carbamazepine and gabapentin. It is important to note that these drugs are analgesic with a central action and, therefore, do not interfere in the nerve damage process, either in its recovery or worsening.

CONCLUSION

This study reveals a considerable prevalence of neuropathic pain among patients with leprosy and presents evidence that this common problem should be a high priority among those in charge of leprosy control programs. In fact, given the present public health policy of shorter regimens and immediate discharge of patients after completion of treatment, this problem could worsen further. Thus, at present there is a strong need to review the concept of leprosy care to provide adequate attention to this disabling complication, and plenty of room for studies on new therapies to cope with this previously ignored clinical problem.

REFERENCES

Pinch Skin Grafting in Non-Healing Leprous Ulcers

Elizabeth Jayaseelan and Vijay V. Aithal

ABSTRACT

Treatment of leprous ulcers has remained inadequate, owing to the fact that most of these ulcers are still being managed conservatively especially in developing nations, probably due to financial constraints. Pinch skin grafting, though obsolete now (2), tries to bridge this gap between cost and effectiveness. It is a simple office-based technique, not requiring much expertise or investment, and can be done in a simple set-up such as a side room (3). Also, pinch skin grafting has an added advantage over single grafts, in that even if one graft is rejected, there are other grafts, which successfully heal, and epidermize to the surrounding. Moreover, if the ulcer is draining, the discharge flows out in between the grafts, thus preventing the whole graft from being rejected. The only disadvantage to pinch skin grafting is the final cosmetic appearance, which might not be most pleasing.

We had very good results with all four patients who underwent this procedure in our institution. The procedure and the final result are described in detail in this report.

RÉSUMÉ

Le traitement des ulcères lèpreux est resté insuffisant, parce que la majorité de ces ulcères est encore appréhendée de manière conservatoire, en particulier dans les pays en voie de développement, qui souffrent de contraintes financières encore importantes. La greffe de peau par pincement, quoique obsolète de nos jours (2), essaye de relier l’incompatibilité entre faible coût et bonne efficacité. C’est une technique simple de pratique courante, ne nécessitant que peu d’expertise et d’investissement, qui peut être réalisée dans une salle de soin (3). De plus, la greffe de peau par pincement présente l’avantage par rapport à la greffe simple d’être formée de plusieurs greffes, si bien que si une d’entre elles est rejetée, la cicatrisation et l’épidermisation peut être assurée par les autres si elles réussissent. Enfin, si l’ulcère est fistulisé dû à un drainage, celui-ci peut s’effectuer entre les greffes, évitant ainsi à toute l’aile greffée d’être rejetée. Le seul désavantage à la greffe par pincement est l’aspect cosmétique final, qui n’est pas toujours très plaisant.

Nous avons eu de très bons résultats chez 4/4 patients qui ont subi cette intervention à notre institution. La procédure et le résultat final sont présentés en détail dans cet article.

RESUMEN

El tratamiento de las úlceras leprosas se ha mantenido inadecuado debido a que la mayoría de éstas todavía se manejan de manera conservadora especialmente en los países en vías de desarrollo, probablemente por dificultades económicas. El procedimiento de injerto de varios fragmentos de piel aunque obsoleto ahora (2), trata de cubrir este hueco entre costo y efectividad. Se trata de una técnica simple que no requiere mucha experiencia o inversión, y que puede hacerse casi en cualquier área adaptada del consultorio (3). El procedimiento de injertos múltiples también tiene la ventaja sobre la aplicación de injertos únicos, de que aun cuando uno de estos injertos se rechace, todavía quedan otros injertos que generalmente sanan exitosamente y epidermizan la zona a su alrededor. Además, si la úlcera está drenando, la descarga fluye entre los injertos, evitando así que estos sean rechazados. La única desventaja de la técnica de injertos múltiples de piel es la apariencia cosmética final, la cual puede ser no muy agradable.

Nosotros hemos tenido muy buenos resultados en 4 pacientes sometidos a este procedimiento en nuestra institución. El procedimiento y el resultado final se describen en este artículo.

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Non-healing ulcers resulting from leprosy pose a great threat not only due to their disability, but also their morbidity. A simple technique like pinch grafting would reduce this morbidity. As compared to single sheet full thickness skin grafts, pinch grafts are easy, inexpensive, and can be done in a minor operating theater, or as an office-based procedure.

Most pinch grafts have been described as split-thickness grafts (2), but they are full-thickness centrally and thin split-thickness at the periphery (3). The procedure involves placing small slices of split skin over the dermabraded ulcer site, and allowing it to heal by epithelialization.

**MATERIALS AND METHODS**

This technique was done on 4 leprosy patients with non-healing ulcers, located at various sites and of differing duration (The Table). All 4 were selected from the leprosy rehabilitation unit of the Dermatology department at St. John’s Medical College Hospital, Bangalore, India.

**Inclusion criteria:** (i) Chronic non-healing ulcers due to leprosy (of more than 2 months duration); (ii) A clean ulcer bed with healthy granulation tissue and good vascularity.

**Exclusion criteria:** (i) Systemic diseases not under control, such as uncontrolled anemia and diabetes mellitus; (ii) Ulcers not primarily due to leprosy.

Serous discharge, though copious, was not a contraindication. Also, those with a hyperkeratotic edge were taken up, after saucerizing the edge, to increase vascularity (Fig. 1)

The donor area was prepared by cleaning the skin with Povidone-Iodine solution (Betadine®) and surgical spirit. The lateral aspect of the mid-thigh was selected as the donor area, although abdomen and buttocks have also been used as donor sites (2). A field block was given by injecting Lignocaine 2%, as so to cover the entire donor area, an area approximately 5 cm × 5 cm for all 10 grafts.

Although various techniques have been described for taking pinch grafts (1, 5), we recommend the shave technique using a hypodermic needle (Fig. 2), which has been described earlier, though some authors consider it obsolete (4). A 23 or 24 gauge needle was inserted into the skin at the donor area, and brought out 1 cm away, so that the skin taken would involve an area of 1 cm². The needle was pushed at a plane, as superficially as possible. The skin was then sliced into a thin layer using a scalpel, just below the needle, at the approximate level of the papillary dermis. This not only ensures minimal blood loss, but also enhances the chance of healing by way of epithelialization. The grafts were then placed in a sterile container containing normal saline and a guaze pad. Adequate numbers of grafts were taken so as to cover the entire ulcer. The donor area was then closed with an antibiotic-impregnated pre-sterilized gauze tulle (Sofratulle®), gauze pads, and an adhesive elastic plaster (Dynaplast®).

The recipient area, usually anesthetic, did not require local anesthetic infiltration. The ulcer bed was dermabraded, using a manual dermabrader. Once the area started bleeding, the grafts were placed over the re-
cipient site, making sure the dermal side faced downwards. The grafts were placed as close to one another as possible (Fig. 3). After the whole area was covered, it was closed with an antibiotic-impregnated pre-sterilized gauze tulle (Sofratulle®), gauze pads, and an adhesive elastic plaster (Dy-naplast®). Joint areas and areas with a high degree of mobility were immobilized in a splint. The dressing was kept for a week, with a change in dressing once every 3 to 4 days. Postoperative care included immobilization, avoidance of wetting the grafted site, a course of antibiotics, analgesics, supplementary zinc, and vitamins.

After a week the dressing was removed, and the grafts looked pinkish-yellow and sodden due to the collection of serous discharge underneath the dressing. It took another week or two for inter-space epithelialization to be completed (Fig. 4). After 4 to 6 weeks, the whole ulcer area was covered by skin.

**RESULTS**

In all 4 patients, the ulcers healed completely (The Table and Fig. 4). Two of the

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/ Sex</th>
<th>Duration of ulcer</th>
<th>Site &amp; area of the ulcer</th>
<th>Duration of leprosy</th>
<th>Treatment received</th>
<th>Time for healing</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42 year F</td>
<td>2 months</td>
<td>Lt. lat. maleolus, 3 cm x 3 cm</td>
<td>18 years</td>
<td>Monotherapy-Dapsone for 2 years Antibiotics, analgesic for the ulcer.</td>
<td>1 month</td>
<td>Immediate response not very good. Gradual healing in 1 month.</td>
</tr>
<tr>
<td>2</td>
<td>49 year M</td>
<td>6 months</td>
<td>Lt. shin. 10 cm x 5 cm</td>
<td>20 years</td>
<td>No treatment for Leprosy. Antibiotics, analgesics for the ulcer.</td>
<td>1½ months</td>
<td>Osteomyelitis (clinically, radiologically, and culture-proved), treated adequately. Anemia corrected. Gradual healing in 1½ months.</td>
</tr>
<tr>
<td>3</td>
<td>38 year M</td>
<td>1 year</td>
<td>Rt. ankle antr. 3 cm x 5 cm</td>
<td>6 years</td>
<td>MDT(?)-2 years. No treatment for ulcer</td>
<td>15 days</td>
<td>Gradual healing in 15 days</td>
</tr>
<tr>
<td>4</td>
<td>40 year M</td>
<td>1 year</td>
<td>Rt. knee antr. 4 cm x 4 cm</td>
<td>10 years</td>
<td>MDT-for 2 years. Antibiotics, analgesics for the ulcer.</td>
<td>15 days</td>
<td>Immobilized the recipient area in a Bohler's iron splint. Gradual healing in 15 days.</td>
</tr>
</tbody>
</table>
patients had ulcers over difficult sites, viz. prepatellar and anterior to the ankle. One of the patients who had underlying osteomyelitis proven clinically and radiologically, and culture-positive for aerobic and anaerobic micro-organisms, also did well under cover of appropriate antibiotics for the osteomyelitis.

**DISCUSSION**

Pinch skin grafting for non-healing ulcers is a simple procedure, which can be done by any doctor with minimal training (1). To the best of our knowledge, this technique has not been previously reported for leprous ulcers. Single sheet split-thickness skin grafts have been described for the treatment of non-healing ulcers; however, pinch grafting seems to have certain advantages over single sheet grafting.

**Advantages of pinch skin graft over single sheet skin graft (split or full thickness)** (1). (i) Pinch grafting can be done in ulcers having unfavorable local factors, such as those with copious serous discharge. If a single sheet of skin is placed in such a situation, the whole graft tends to get thrown off, due to collection of the discharge underneath. In contrast, in pinch grafts the discharge is allowed to drain from the inter-space. (ii) Even if one graft gets rejected, the other grafts would still take up and the rejected area would epithelialize from the adjacent grafts. (iii) This simple technique can be done by anyone, with little training and experience. (iv) The set-up required is very simple and inexpensive.

**Disadvantages** (5). The only disadvantage is that it results in cobbling, scarring, and depigmentation (1).

**CONCLUSION**

Pinch skin grafting is a safe, simple, and inexpensive technique, which can be easily mastered, with minimal training and experience. We recommend it to be used in all uncomplicated leprous ulcers, which cannot be brought under control with drugs and dressing alone. In the future, we plan to extend this study to include pinch grafting on plantar ulcers.

**REFERENCES**

Single Nucleotide Polymorphisms (SNPs) at -238 and -308 Positions in the TNFα Promoter: Clinical and Bacteriological Evaluation in Leprosy


ABSTRACT

Tumor necrosis factor alpha (TNFα) plays a key role in orchestrating the complex events involved in inflammation and immune response. The presence of single nucleotide polymorphisms (SNPs) within the promoter region of the TNFα gene has been associated with a number of diseases. The aim of this study was to investigate the distribution of polymorphisms at positions -238 (G/A) and -308 (G/A) at the TNFα promoter, and its association to the outcome of different clinical forms of leprosy. Furthermore, the bacteriological index (BI) was evaluated among genotyped multibacillary (MB) patients in order to investigate the possible influence of each polymorphism on the bacterial load. This study included a total of 631 leprosy patients being 401 MB and 230 paucibacillary (PB), that was further separated according to its ethnicity (Afro- and Euro-Brazilians). The combination of SNPs in haplotypes generated three different arrangements: TNFG-G, TNFG-A and TNFA-G. In spite of the marked differences observed in the frequency of the haplotypes along the ethnic groups, no statistical differences were observed in haplotype frequencies between MB and PB patients. The BI analyses showed a lower bacteriological index among the -308 carriers, while the BI of the -238 carriers was higher. Although no significance has been achieved in this analysis regarding the influence of the polymorphisms to the development of the clinical outcome, it seems that in a different stage (among the MB patients) the polymorphisms could contribute to the degree of severity observed.

RÉSUMÉ

Le facteur alpha de nécrose tumorale (TNFα) joue un rôle important dans l’ajustement des évènements complexes qui régulent l’inflammation et la réponse immunitaire. La présence de polymorphismes mono-nucléotidiques (SNPs) au sein de la région promotrice du gène codant pour TNFα a été associée à un certain nombre de maladies. Le but de cet article était d’explorer la distribution de polymorphismes aux positions -238 (G/A) et –308 (G/A) du promoteur de TNFα et son association aux résultats phénotypiques des différentes formes de leprose. De plus, l’index bactérioscopique (IB) a été évalué parmi les patients multibacillaires (MB) génotyptés dans le but d’évaluer la possible influence de chaque polymorphisme sur la charge bactérienne. Cette étude a porté sur 631 lépreux comportant 401 MB et 230 PB, qui furent encore séparés par ethnie (Afro et Euro-brésiliens). La combinaison des SNPs en haplotypes a généré 3 arrangements différents : TNFG-G, TNFG-A et TNFA-G. En dépit de différences marquées observées dans les fréquences haplotypiques entre les groupes ethniques, aucune différence statistiquement significative ne fut observée entre les patients MB et PB. Les analyses de IB ont montré un index bactérioscopique plus faible parmi les porteurs –308, tandis que le IB des porteurs -238 était plus élevé. Bien que cette analyse de polymorphismes n’ait pas démontré de différence significative sur l’issue clinique de la
Leprosy, a chronic human disease, is the result of infection by *Mycobacterium leprae*. The clinical spectrum of the disease includes two polar (lepromatous-LL and tuberculoid-TT) and three borderline forms (tuberculoid-BT, borderline-BB, and lepromatous-BL) (19). The multibacillary (MB) form, including LL, BB, and BL, is characterized by low immune responsiveness and high bacterial load. The paucibacillary (PB) form, including TT and BT, is marked by strong cell-mediated immunity against the bacillus (22). Much evidence has implicated cytokines in the immune response of leprosy (16), mainly the tumor necrosis factor-alpha (TNFα), which has a beneficial function in host defense but, if produced in high levels, contributes to tissue damage (25).

The presence of single nucleotide polymorphisms (SNPs) in the TNFα promoter region and their association with autoimmune and infectious diseases has been extensively studied (10). The polymorphism detected at position -308 (G/A) within the promoter region of the TNFα gene (29) was the first one found to be associated with disease (18). This polymorphism has been observed in association with several other infectious diseases where excessive TNFα production seems to play a role, such as in cerebral malaria (12) and mucocutaneous leishmaniasis (7). An association of the -308 polymorphism with the development of lepromatous leprosy was previously reported in an Indian population (21). However, in Brazilian leprosy patients the association of this polymorphism with protection has been described (23, 24, 27). A plausible explanation for these divergent findings may involve the ethnicity, where the allelic frequency of TNFα-308A in leprosy patients range from 7.0 to 10.8 in Indian and Brazilian, respectively (21, 24). This allelic frequency variation is wide among populations such as Caucasian Irish (23.0), African Zulu (22.1), Arabian Omani (8.1), Singapore Chinese (12.0) and Mexican Mestizos (2.5) (13).

Studies at another G/A transition polymorphism (-238) in the TNFα promoter region (4) has shown that the A allele was increased among patients with chronic hepatitis B and C, suggesting association to disease susceptibility (7, 8) while in cancer a protective effect was observed (7). In fact, the complex relationship between SNPs in the human genome and disease association indicates the need for the construction of haplotypes (specific combination of SNPs on the same chromosome) on the locus studied because they are more informative than any single SNP (1, 26).

In the recent analyses from Brazilian leprosy patients we have shown in paucibacillary forms an increased allelic frequency of the TNF-308A in comparison to the multibacillary (0.14 and 0.09, respectively) with a borderline significance ($\chi^2 = 3.47; p = 0.06$) (24). This data did not define if TNFα...
-308A was a trend marker for protection, i.e., allele frequency in control > PB > MB or whether TNF-308A discriminates between cases and controls only, being a resistance locus of leprosy per se. Thus, the aim of this study was to describe whether there was a difference between PB and MB using an increased number of patients. To overcome a possible cryptic stratification that would impact the SNP frequency and mask an association effect, patients were separated according to ethnicity. Besides, the -308 and -238 SNPs were combined in haplotypes that better analyze the TNF promoter region. In addition, to understand the impact of polymorphisms of TNFα promoter in relation to progression of the disease, the comparison between SNPs in -308 and -238 position and the bacteriological index (BI) was set out in MB patients.

**MATERIALS AND METHODS**

**Patients.** Six hundred and thirty one leprosy patients from the Leprosy Out-Patient Unit, Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) were included in this study. They were diagnosed on the basis of clinical and bacteriological criteria and classified according to the Ridley and Jopling Scale (20). Four hundred one MB and 230 PB patients were studied, including 405 males and 226 females. Brazilians were classified according to their ethnic origin after careful inspection of facial morphological features, hair type and skin color. Two groups were ascertained: Afro-Brazilians and Euro-Brazilians with N = 251 and N = 235, respectively. Asians and Amerindians are not commonly represented in the population of Rio de Janeiro and were not observed among the individuals inspected.

Bacteriological index determination. Bacteriological index (BI) was determined according to Ridley, 1964 (19) among the multibacillary patients in slit skin smears from six different anatomic sites and ranged from 0.16 to 5.33 (mean = 2.50 ± 1.46).

**DNA extraction and SNPs genotyping.** Genomic DNA was prepared from frozen whole blood collected with sodium citrate buffer by a commercially available DNAzol extraction kit (Invitrogen Life Technologies, Gaithersburg, MD, USA). Genotyping of the TNFα promoter region for analysis of polymorphisms at the -238 and -308 positions was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP), a single PCR step and further restriction analysis (6, 29). Restricted amplified products were visualized by electrophoresis in 3% agarose gel and ethidium bromide staining.

**Statistical analysis.** The statistical significance of TNFα promoter polymorphism distributions was analyzed by way of the χ2 test Odds-ratio (OR) and 95% confidence intervals (CI). Yates’ correction or Fisher’s exact test was used when appropriate (Epi Info 6: CDC, Atlanta, GA). The significance level adopted was p <0.05. The haplotype frequencies were estimated using the EH program (28).

**Generalized additive model.** the variability of the bacteriological index (BI) in relation of the presence of the mutation in the TNFα promoter was studied through a generalized additive model (GAM). The GAM is a special regression model, in which some of regression assumptions are relaxed. So, GAM models may have advantages in many biological phenomena, and may replace traditional linear or logistic regression models. In mathematical terms, GAM models allow predictors (or covariates) to be replaced by arbitrary smooth functions. For instance, B-splines (polynomials that can be adjusted to a set of points) could be used as a smoothing function. In this context, a GAM model was used to study the association between the occurrence of the G/A substitution in the positions -308 and -238 of the promoter gene of TNFα, and the baciloscopic index, calculated as previously described. For the adjustment of the model, one should indicate the number of nodes necessary. A node is the number of points used for the curve to be adjusted. The SPLUS 2000 program, Professional Release 3, MathSoft, Inc. was used to perform these analyses.

**RESULTS**

**Analyses of TNFα haplotypes frequencies in leprosy patients in different Brazilian ethnic groups.** Haplotypes of TNFα were estimated using a maximum-likelihood probability test from -308 and -238 SNP genotypes of unrelated patients stratified in Euro- and Afro-Brazilian (The Table). Three different haplotypes on the TNFα gene have been identified (TNFG-G, TNFA-G, TNFG-A). No statistical differ-
ences were observed in haplotypes from TNFα promoter between MB and PB patients irrespective to the ethnic group analyzed. The TNFG-G haplotype in both PB and MB forms presented the highest frequency among patients in both ethnic groups. Marked differences between Euro- and Afro-Brazilians were observed when other haplotypes were analyzed. Among PB patients, an increased frequency of the TNFA-G haplotype in Euro-Brazilian was detected in comparison to Afro-Brazilian (0.16 and 0.08, respectively) was detected.

**Analyses of the bacteriological index (BI) from multibacillary patients genotyped for SNPs at position -308 and -238.** Bacteriological index (BI) variability in relation to the presence of the -238 (N = 343) and -308 (N = 341) polymorphisms was analyzed via the GAM (Fig. 1A, B). The GAM regression model was performed to study the association between the presence of the A allele at the positions -238 and -308 of the promoter gene of TNFα, as the dependent variable, and the variability of the bacteriological index, as a predictor variable. For the model using the substitution at position -238, a regression polynomial spline with 1.5 nodes was used. It was observed that the probability for the occurrence of the A allele at the -238 position increases with higher bacteriological index (Fig. 1A).

For the model using the substitution at position -308, a regression polynomial spline with 1.1 nodes was used. In contrast to the result obtained with -238, the probability for the occurrence of the A allele at the -308 position is greater with a low BI (Fig. 1B).

**DISCUSSION**

Studies using the frequency of single nucleotide polymorphisms in candidate genes are interesting approaches to the investigation of the susceptibility and severity of diseases. It is believed that SNPs are relevant since they can be used as genotypic markers of specific disease phenotypes or can regulate biological phenomena influencing mRNA expression, thereby altering mRNA isoforms (unravelling cryptic splicing sites) or modifying enzymatic activity of genes (11). The problem is that SNP frequencies vary enormously among populations (13), especially Brazilians who originated from a variety of ethnicities, mainly Portuguese explorers mixed with native Amerindians and Africans (3). The outcome of this admixed colonization is a dense population without a clear genetic/morphological ethnic cut off (17). However, some cryptic stratification may still be functioning as confounding factors in Brazilians in population-based studies. Indeed, the separation according to the morphological features of the patients better discriminate Afro- and Euro-Brazilians, where a difference in the -308A/-238G haplotype frequency from Afro-Brazilian (9%) to Euro-Brazilian (13%) patients was observed. Still, no statistical differences were observed when PB and MB were compared, demonstrating that if there is some cryptic stratification due to admixture, it is not being detected by conventional morphological inspection in our patient population. Thus, to scrutinize the stratification in Brazilian population-based studies it would be necessary to use genomic controls (5). Moreover, a recent study performed in Gambian and Malawian populations studying SNPs spanning 4.4kb of the TNFα/LTα locus demonstrated the need to Type 8 in Gambians, and 7 out 12 SNPs in Malawians, to detect the haplotypic structure and informative SNPs in this region due to the high frequency of recombination (1). We do not have data for the Brazilians but it seems to be necessary to enlarge the focused region of the TNF locus to capture more information about severity in leprosy.
On the other hand, the analysis of BI and its association with polymorphisms at positions -308 and -238 in TNFα suggested these polymorphisms are functionally relevant. We previously demonstrated that TNFα -308A was associated with a stronger response in Mitsuda reaction (15). In this study, GAM analyses in -308A revealed that such patients have lower BIs. The results of our previous findings (15) with this new data is that TNF -308A could be upregulating the secretion of TNFα that, in turn, induces a stronger DTH skin response in paucibacillary patients and restricts M. leprae growth in multibacillary patients.

The opposite was verified concerning the study of -238A and BIs. In this case, the presence of the A allele was more frequent in multibacillary patients with higher BIs. This data is in accordance with the literature, where it has been demonstrated that the -238A polymorphism is associated with lower levels of TNFα (10).

The possibility of using slit skin smears is one of the few alternatives for in vivo determination of the bacterial load. The BI is one of the clinical parameters indicating disease progression and severity, representing a clear risk factor for the development of the acute inflammatory episodes in leprosy (14). Thus, by way of the adjusted model (GAM), the existence of a clinical significance for the variability of BI in relation to the presence of polymorphisms in the TNFα promoter suggests a functional dichotomy between the -308 and -238 SNPs in relation to TNF regulation and leprosy progression.

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Leprosy Reactions: Humoral and Cellular Immune Responses to *M. leprae*, 65kDa, 28kDa, and 18 kDa Antigens

Keshar K. Mohanty, Beenu Joshi, Kiran Katoch, and Utpal Sengupta

ABSTRACT

This study examines the immune responses against some stress proteins of *Mycobacterium leprae* in leprosy patients with and without leprosy reactions. Leprosy patients showed a higher level of antibodies to all antigens compared to healthy controls. The antibody response to 18kDa antigen was significantly higher in patients with Type 1 reaction compared to those of TT or borderline patients without Type 1 reaction, or those with Type 2 reaction. Borderline (BT/BL), lepromatous (LL) and patients with reactions (Type 1 and Type 2) had higher levels of antibodies to *M. leprae* soluble extract (MLSE) and 65kDa than those of the tuberculoid (TT) group. LL, borderline patients, and patients with Type 1 reaction had a higher level of antibody to 28kDa than those of healthy controls. However, no significant differences could be observed in antibody response to these antigens (MLSE, 65kDa, and 28kDa) between patients with reaction and without reaction. A significant proportion of TT/BT patients showed positive lymphoproliferative response to MLSE compared to BL/LL patients. In addition, the lymphoproliferative response to MLSE was significantly greater in patients with Type 1 reaction compared to patients without reaction. No difference in proliferative response to 65kDa could be observed in any of these groups. The finding of high levels of antibodies against stress proteins in patients with Type 1 reactions, especially to 18 kDa antigen, along with a heightened lymphoproliferative response to MLSE is suggestive of a coexistence of cell mediated and humoral immunity in leprosy patients during Type 1 reactions. On the other hand, in Type 2 reactions no significant role of stress proteins could be demonstrated except a heightened lymphoproliferative response to the 28 kDa antigen.

RÉSUMÉ

Cette étude présente les réponses immunitaires chez les patients hanséniens et les patients souffrant de réactions, contre les protéines de stress de *Mycobacterium leprae*. Les patients hanséniens ont montré de plus haut niveaux d’anticorps dirigés contre tous les antigènes que les personnes témoins en bonne santé. La réponse sérique dirigée contre l’antigène de 18kDa était significativement plus élevée chez les patients souffrant de réaction de type 1 comparée à celles des patients TT ou borderline, ou celle des patients avec réaction de type 2. De plus, un plus grand pourcentage de patients avec réaction inverse avaient une réponse détectable pour cet anticorps, comparé à celui des patients sans réaction. Les patients borderline (BT/BL), lépromateux (LL) et les patients avec réactions (type 1 et type 2) présentaient de plus hauts niveaux d’anticorps dirigés contre l’extrait soluble de *M. leprae* (MLSE) et la protéine 65kDa que les patients tuberculoides (TT). Les patients LL, borderline et les patients présentant une réaction de type 1 présentaient de plus haut niveau d’anticorps contre la protéine 28kDa par rapport aux témoins en bonne santé. Cependant, aucune différence significative ne fut observée entre les patients avec et sans réaction dans la réponse sérique contre les antigènes MLSE, 65kDa et 28kDa. Comparé aux patients BL/LL, une proportion significative de patients TT/BL montraient une réponse lymphoproliférative positive contre MLSE. De plus, comparé aux patients sans réaction en cours, les patients de type 1 montraient une réponse lymphoproliférative plus élevée contre MLSE. Aucune différence de réponse proliférative contre 65kDa ne fut observée entre les groupes. La mise en évidence de hauts niveaux d’anticorps dirigés contre les protéines de stress de *M. leprae* chez les pa-

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Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. After infection the response of host immune system determines the course of the disease. On the basis of cellular immune response of the host, a spectrum of types of leprosy has been described (27). Some types of leprosy patients suffer from immunological complications known as “lepra reactions” which include both Type 1 (reversal reactions, RR) and Type 2 (erythema nodosum leprosum, ENL) reactions (14). Type 1 reaction is characterized by episodes of increased inflammatory activity in skin and in nerves of patients with borderline leprosy [Borderline tuberculoid (BT), Borderline (BB), and Borderline lepromatous (BL)] (5, 31). Type 2 reaction or ENL reaction is the most serious immunological complication in BL and lepromatous (LL) patients. Type 1 reaction is known to be due to changes in (both up and down regulation) of cell mediated immunity (CMI), whereas in Type 2, there is a rise in immune complexes with their deposition in tissues (40). Further, in Type 2 reactions a transient rise of CMI with the expression of Th 1 type of cytokines has also been noted (19, 35).

Several stress proteins of *M. leprae* have been cloned (42) and are recognized by both murine and human immune cells (22, 24). *M. leprae* heat shock protein (hsp) 65 kDa, expressed in macrophages transfected with the mycobacterial gene, is known to be presented to T cells in association with both MHC class I and class II (32) and also MHC non-restricted manner (33). Beimnet, et al. (1996) also reported the expression of Hsp 60 from a human monocytic cell line infected with *M. leprae* (2). Further, these stress proteins are known to be major antigens of mycobacteria, which induce specific antibody and T cell immune response during infections (1, 6, 8, 10, 11, 14, 16, 21, 28, 29, 30, 37, 41).

Nerve and skin damage in leprosy is reported to be associated with increased lev-
els of intra-lesional hsp (16). The process of reaction evokes a change in immunological status of the host leading to stress conditions for bacteria, which might result in release of stress proteins. However, the role of antibodies to stress proteins in Type 1 reactions has not been elucidated so far except for the observation of Klatser, et al. (17). To explore the immunological role of stress proteins of M. leprae in leprosy, analysis of circulating antibodies, and of the proliferative response of peripheral blood mononuclear cells (PBMC) to some of the stress proteins were performed in healthy controls, Tuberculoid (TT), Borderline and LL patients with Type 1 or Type 2 reactions, and in patients without reactions.

MATERIALS AND METHODS

Leprosy patients attending the out patient department of the Central JALMA Institute for Leprosy (Agra, India) were included in the study after obtaining their written consent according to the guidelines laid by the Indian Council of Medical Research, India. They were diagnosed clinically and bacteriologically and were divided into five groups across the disease spectrum according to the criteria of the Indian association of Leprologists (12). All these patients were clinically active and were on multi-drug therapy (MDT) during the study period.

Serum samples. Serum samples were obtained from 10 ml of blood drawn by antecubital venipuncture from 6 TT, 24 borderline (13 BT, 11 BL), 8 LL patients who were stable in their clinical manifestations, 21 BL/LL patients with ENL, and 29 BT/BL patients with Type 1 reaction. Nineteen laboratory volunteers who were hospital contacts served as healthy controls.

Soluble and recombinant antigens of M. leprae. M. leprae soluble extract (MLSE) (contract No-1-A1-55262) was obtained from Dr. P. J. Brennan, Colorado State University, U.S.A., and the recombinant proteins of M. leprae (ML hsp65kDa, ML 28 kDa, ML 18kDa) were gifted by Dr. M. Singh, GBH, Germany.

Enzyme linked immuno sorbent assay (ELISA). Maxisorp (Nunc, Roskilde, Denmark) plates were coated with 100 microlitre (µl) antigen solutions [MLSE (2µg/ml), 65 kDa (1µg /ml), 28 kDa (2µg/ml) and 18 kDa (2µg/ml)] in carbonate bicarbonate buffer (pH 9.2) and kept for 4 hrs at 37°C and then overnight at 4°C. The wells were blocked in phosphate buffered saline (PBS) with 3% Bovine Serum Albumin (BSA) for 1 hr at 37°C. One hundred µl of 100-fold diluted sera in PBS containing 0.05% Tween 20 and 1% BSA was added in duplicate wells. After 2 hrs incubation at 37°C, the plates were washed 3 times in PBS Tween (0.05%). One hundred µl of 5000-fold diluted horseradish peroxidase-conjugated anti-human IgG antibody (Sigma, St. Louis) was added, and plates were incubated for 1 hr 30 minutes at 37°C followed by a final 3 washes. One hundred µl of orthophenylene diamine hydrochloride solution (0.5 mg/ml substrate in distilled water containing 30 µl of 30% H₂O₂) was added to each well for development of color. The reaction was stopped after 30 minutes by adding 25 µl of 3N H2SO₄. The optical density (OD) was measured in an ELISA reader at 492 nm.

Peripheral blood mononuclear cells (PBMCs). Peripheral venous blood was collected aseptically in a heparinized tube from 2 TT, 22 borderline (10 BT, 12 BL), 7 LL patients who were stable in their clinical manifestations and 12 BL/LL patients with Type 2, 12 BT/BL patients with Type 1, and from 12 healthy controls. PBMCs were separated by ficoll hypaque density gradient centrifugation. The cells were collected from the interface layer and washed three times with RPMI 1640 and counted in a Neubaur’s chamber.

Proliferation of peripheral blood mononuclear cells (Lymphocyte transformation test, LTT). PBMCs (2 × 10⁶ cells/ml) were cultured in quadruplicate wells in RPMI 1640 containing 10% human AB serum in 96-well plates (Nunc, Roskilde, Denmark) for six days. Optimum concentrations of MLSE (1 µg/ml) and different recombinant proteins [65kDa (5µg/ml), 28 kDa (10 µg/ml)], were added to wells for sensitization of PBMCs. DNA synthesis was assayed by [³H] labeled thymidine incorporation (Amersham, U.K.). 1 µ Ci of [³H]-thymidine (specific activity 5.0 Ci / m mol) was added to each well on 5th day and after 18 hr cells were harvested. The lymphocyte stimulation index (SI) was calculated using a standard formula (average cpm in the presence
Table 1. Mean level of antibodies (with standard deviations) to antigens of M. leprae of healthy controls and leprosy patients.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>HC (N = 19)</th>
<th>TT (N = 6)</th>
<th>Borderline (BT/BL) (N = 24)</th>
<th>LL (N = 8)</th>
<th>ENL (N = 21)</th>
<th>RR (N = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSE</td>
<td>0.23 ± 0.13</td>
<td>0.30 ± 0.06</td>
<td>0.51 ± 0.36( ^v )</td>
<td>0.55 ± 0.19( ^v )</td>
<td>0.58 ± 0.37( ^v )</td>
<td>0.51 ± 0.22( ^v )</td>
</tr>
<tr>
<td>ML65kDa</td>
<td>0.19 ± 0.08</td>
<td>0.22 ± 0.04</td>
<td>0.29 ± 0.13( ^v )</td>
<td>0.37 ± 0.16( ^v )</td>
<td>0.30 ± 0.13( ^v )</td>
<td>0.35 ± 0.22( ^v )</td>
</tr>
<tr>
<td>ML28kDa</td>
<td>0.17 ± 0.08</td>
<td>0.30 ± 0.17</td>
<td>0.27 ± 0.13( ^* )</td>
<td>0.42 ± 0.25( ^* )</td>
<td>0.29 ± 0.13( ^* )</td>
<td>0.38 ± 0.19( ^* )</td>
</tr>
<tr>
<td>ML18kDa</td>
<td>0.17 ± 0.08</td>
<td>0.23 ± 0.06( ^* )</td>
<td>0.28 ± 0.15( ^* )</td>
<td>0.42 ± 0.19( ^* )</td>
<td>0.31 ± 0.22( ^* )</td>
<td>0.46 ± 0.35( ^* )</td>
</tr>
</tbody>
</table>

\( ^v \) Significantly more than HC & TT (p <0.005)

\( ^* \) Significantly more than HC & TT (p <0.02)

\( ^* \) Significantly more than HC (p <0.0005)

\( ^v \) Significantly more than Borderline (BT/BL) and ENL (p <0.03)

\( ^* \) Significantly more than HC (p <0.05)

\( ^* \) Significantly more than TT, Borderline (BT/BL) and ENL (p <0.005)

Table 2. The percentage of seropositivity for antibodies against various antigens of M. leprae.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>HC (N = 19)</th>
<th>TT (N = 6)</th>
<th>Borderline (BT/BL) (N = 24)</th>
<th>LL (N = 8)</th>
<th>ENL (N = 21)</th>
<th>RR (N = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLSE</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>10 (41.66)</td>
<td>5 (62.5)</td>
<td>10 (47.61)</td>
<td>15 (51.72)</td>
</tr>
<tr>
<td>ML65kDa</td>
<td>1 (5.26)</td>
<td>0 (0)</td>
<td>8 (33.33)</td>
<td>4 (50)</td>
<td>6 (28.57)</td>
<td>11 (37.93)</td>
</tr>
<tr>
<td>ML28kDa</td>
<td>0 (0)</td>
<td>2 (33.33)</td>
<td>8 (33.33)</td>
<td>4 (50)</td>
<td>7 (33.33)</td>
<td>15 (51.72)</td>
</tr>
<tr>
<td>ML18kDa</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (29.16)</td>
<td>5 (62.5)</td>
<td>5 (23.80)</td>
<td>16 (55.17)</td>
</tr>
</tbody>
</table>

Figures in parentheses () show percent positive.

Cut off values are mean OD +2 S.D. (MLSE = 0.49), (65kDa = 0.35), (28 kDa = 0.33), and (28 kDa = 0.33). Individuals having more OD values than cut off points are taken as positive.

* Significantly more than TT (p <0.03)

* Significantly more than borderline group (p <0.06)
significantly higher level of antibody than did TT patients. The mean antibody level to this antigen is highest in BT/BL patients during Type 1 reactions, which is significantly different from those of TT patients, BT/BL patients without reaction and patients with Type 2 reaction, (p < 0.005).

The percentage of seropositivity of antibodies to these antigens is shown in Table 2. It was noted that significant proportion of LL patients showed positivity for MLSE and 18kDa (p <0.03) when compared to that of TT patients.

There was no significant difference in seropositivity of antibodies to these antigens amongst patients of the BT/BL groups with and without reaction. Nevertheless a significant proportion of BT/BL patients with Type 1 showed higher positivity to 18-kDa antigens only when compared to those of borderline patients without reactions (p <0.06).

**Lymphoproliferative response to MLSE and stress proteins of M. leprae (ML 65kDa & ML28kDa).** The positivity for lymphoproliferation to these antigens (SI ≥2) in healthy controls and leprosy patients is presented in Table 3. The individual lymphoproliferative responses against these antigens are shown in The Figure (a, b, and c).

**MLSE.** MLSE was found to be the best inducer for proliferation of lymphocytes amongst all M. leprae proteins tested. All except one of the healthy controls showed a positive response to MLSE, whereas 1 BL patient, 1 BL patient with Type 2 reaction and none of the LL patients, were positive to this antigen. 8/12 (66.6%) of Type 1 reactions, and 6/12 TT/ BT (50%) were positive to these antigens. The mean proliferative response of BT/BL patients with Type 1 was significantly greater compared to borderline patients without reaction (p < 0.02). However, there was no difference in mean proliferative response between BL/LL without reactions and Type 2 reaction patients. Further, the mean proliferative response was significantly greater in TT/BT patients compared to BL/LL patients (p < 0.03) and Type 2 reaction patients (p < 0.04). Significant proportions of TT/BT individuals showed lymphoproliferation when compared to BL/LL patients (p < 0.007).

**Response to recombinant proteins.** ML 65kDa. There was no significant difference in lymphoproliferative response to 65kDa protein amongst these groups (Table 3 and The Figure b). ML 28kDa. While 3/12 patients with Type 2 showed a positive response, none of the LL patients was responsive to this antigen (Table 3 and The Figure c).

**DISCUSSION**

When infectious agents enter the host, they may respond to the host environment by producing stress proteins. These stress proteins are important in eliciting immune response, which can lead to pathogenesis or protection in the host. Some recombinant antigens (stress proteins) have been reported to be immunologically important and induce B cell and T cell immune responses in leprosy (6, 8, 16, 21, 24, 30, 37, 38, 41). Although these previous studies have been conducted to analyze the immune response in leprosy patients, only a few studies were carried out in leprosy patients with reactions. The objective of the present study was to analyze the level of antibodies and immunoproliferative response of PBMCs to MLSE and a few stress proteins of M. lep-
in patients associated with reactions, and to compare their levels with patients who are not associated with reactions. In addition, as controls, responses of some healthy individuals who were exposed to infection in the hospital were also compared with these leprosy patients.

Although the mean antibody level was found to be highest in patients with Type 2 reactions, this was not significantly different from other groups. Patients with Type 1 reactions also showed almost the same level of antibody to MLSE as of borderline patients. The mean level of antibodies to
MLSE was found to be significantly higher in all types of leprosy patients except TT patients when compared to those of healthy individuals. Among the patient groups, TT patients showed lowest antibody level. The mean OD value gradually increased from the TT end to the LL end of the spectrum. A similar finding has been reported earlier by Qin-xue, et al. (26). This finding of a gradual increase of antibody level against MLSE could possibly be due to the increase in antigenic load from the TT pole to the LL pole.

An increased level of antibodies was seen to 65 kDa, 28kDa and 18 kDa stress proteins in all groups of leprosy patients compared to healthy individuals, similar to previous reports (14, 16). In our study a higher level of anti 65kDa antibody was observed in BL/LL and Type 2 patients. However, there was no significant difference in antibody levels between patients with reaction and patients without reactions. Possibly the 65kDa antigen induces an antibody response in the initial phase of infection and this does not change during the development of various stages of disease. This observation would suggest that antibodies to 65kDa do not induce any immunopathological phenomenon in patients associated with reactions. Furthermore, the above finding is consistent with the observation of Thole, et al. (1995) who did not find any association with the 65kDa antigen specific responses in BT/TT or LL types of leprosy (37). Of course, M. leprae 65 kDa has been noted to be expressed in skin and nerve of all groups of leprosy patients (38) and may be presumed to have an important role in Type 1 reaction, but it is uncertain whether this is predominantly related to the initiation of the disease or the development of disease once the reaction has started.

We observed a higher positivity for antibody responses against the 28 kDa antigen in LL patients than TT patients, and this response was even greater in patients with Type 1 reactions. Though other studies have provided evidence of the presence of anti M. leprae 28 kDa antibodies in sera of lepromatous patients (6, 16), this is the first report to note such a higher percentage (51.72%) of antibody positivity to this stress protein of M. leprae in patients during Type 1 reactions. This antigen has recently been suggested as a potential candidate antigen for initiating the Type 1 reaction, because it has been demonstrated in macrophages and Schwann cells of skin and nerve biopsies (20). Hence, our finding of high antibody response may be due to expression of this antigen by M. leprae during Type 1 reactions. Interestingly, the M. leprae 28 kDa protein is known to have a sequence similarity with human superoxide dismutase (SOD) (67%) and E. coli SOD (55%) (36). The elevated antibody level to 28kDa antigen in some of the LL and borderline patients with Type 1 reaction may be attributed to the response against increased expression of SOD in response to environmental stress during the disease process or during reaction.

The most interesting finding of our study is that the antibody level against M. leprae 18kDa antigen was much higher in leprosy patients with Type 1 reactions, although the percent seropositivity was also high in LL patients without reactions. Our study indicates that production of anti18-kDa antibody is a prominent event in leprosy, as all groups of leprosy patients except TT patients had a high level of antibodies to this antigen. Khan, et al. reported a low reactivity to this antigen in multibacillary patients (15), but we observed seropositivity of 62.5% in LL patients. Further, Roche, et al. (1991) observed a similar finding of a low level of anti 18kDa antibodies in paucibacillary (PB) patients and a high level in multibacillary (MB) patients (28). Many other authors have described the 18kDa protein as one of the important antigens which produces significant B cell and T cell immune response in leprosy (8, 10, 11, 24, 28). However, its association with Type 1 reactions has not been described previously. This protein was reported to have strikingly similar size and sequence to a family of heat shock proteins (25) and is expressed during heat stress (19). So, we postulate that the expression of this antigen by M. leprae might be increasing due to cellular resistance by the host, and as a result the host responds by producing antibodies to this stress protein. This could induce an immune response in the initial phase of infections and during Type 1 reactions.

From our observations, we conclude that circulating antibodies to some of the stress
proteins of *M. leprae* appear to play a role in Type 1 reactions. We could not observe any significant difference in antibody level against the recombinant proteins in LL patients, nor in patients with Type 2 reaction, though the reactivity was greater to some antigens in patients with Type 2 reactions than TT patients or healthy controls. Miller, *et al.* (1984) have also reported that the occurrence of Type 2 reaction had no significant effect on the total level of IgG antibody against arabinomannan (23).

With regard to the lymphoproliferative responses to these antigens, all healthy individuals except one responded to MLSE, but none of the recombinant proteins induced a strong proliferative response in this group, confirming the earlier report of Wilkinson, *et al.* (41). Moreover, most of the patients and all healthy controls were responsive to the purified protein derivative of *M. bovis* (data not included). In the present study, TT/BT patients showed stronger lymphoproliferative responses than those of BL/LL patients only to MLSE and not to recombinant proteins of *M. leprae*, consistent with the study of Thole, *et al.* (37) Further, we observed a significant lymphoproliferative response to MLSE in patients with Type 1 reaction. Bjune, *et al.* (5) have already noted this with sonicated preparations of *M. leprae* in patients with Type 1 reactions. The finding of a significant lymphoproliferative response to MLSE during Type 1 reactions, compared with borderline patients without reactions, clearly indicates the upregulation of CMI in such patients.

We did not observe any significant difference in the proliferative response to 65 kDa antigen among patient groups, as reported by others. Ilangumaran, *et al.* (13) reported that there is an inverse relationship between cell mediated and humoral immune responses to 65 kDa in leprosy patients. De La Barrera, *et al.* (7) have observed that *M. leprae* 65 kDa is a poor inducer of cytotoxic T lymphocyte (CTL) in MB patients, but could induce proliferation and CTL in MB patients with Type 2 reaction.

The finding that the 28 kDa antigen induced a poor response both in TT and LL patients is in agreement with the study of Wilkinson, *et al.* (41). Lepromatous patients did not differ significantly from patients with reaction in their proliferative response to all recombinant proteins tested in our study except that of the 28 kDa antigen where a significant proportion of patients with Type 2 reaction responded to this antigen. While a number of studies have reported the antigenic potential of 28 kDa in the humoral immune response, not much information is available regarding the nature of cell mediated response against it. Though Wilkinson, *et al.* (41) have described this as a moderate stimulator of T cell responses, they did not investigate the response in patients during leprosy reactions (Type 1 or Type 2). The significantly greater positivity in proliferative responses in Type 2 patients than those of BT/BL/LL might indicate a response to the expression of SOD during this reactional stress. The finding of a significantly greater number of Type 2 reaction cases responding to the 28 kDa antigen compared to BL/LL patients might explain their transient boost in CMI as reported earlier by other authors (19).

Although previous workers have demonstrated the presence of antibodies to these proteins in BT/TT and BL/LL patients, this appears to be the first study to demonstrate a high level of antibodies especially against 28 kDa in these patients associated with Type 1 reaction. The role of *M. leprae* antigens in Type 1 reactional pathology has been noted others (17, 20, 39). Hence, the high level of antibodies observed in patients during Type 1 reaction may be due to the *M. leprae* antigens exposed in tissues during reactions. At this moment, it is not possible to conclude whether antibodies are induced due to the development of reactional pathology, or if it has been initiated due to the induction of antibodies. The cellular immune response associated with Type 1 reaction is presumably due to other *M. leprae* antigens and not due to the stress proteins expressed by *M. leprae*.

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Axonal Spherical Bodies in the Peripheral Nerves of Leprosy Patients

Mutsuhiro Furuta, Kentaro Hatano, Yoshiko Okano, Takanobu Matsuki, Takeshi Ikeda, Kouichi Nakatani, Atsuo Sato, and Mutsue Mizushima

ABSTRACT

Spherical bodies, roughly 10 µm in diameter, which have not been reported before, were found in the peripheral nerve axons of specimens collected during post-mortem examination of leprosy patients.

These bodies were found in the fascicles of all peripheral nerves of the extremities examined (median, radial, ulnar, peroneal and sciatic nerves). Their incidence was not related to the type of leprosy. The area immediately below the thickened perineurium, a feature associated with leprosy, often showed a large number of spherical bodies.

When observed under a transmission electron microscope, the spherical lesions often showed a lamellar structure, although some of them were amorphous. No structure resembling organelles was seen within the bodies.

Observation with the merge technique showed a clearly lamellar structure in most of the spherical bodies. These bodies and the surrounding myelin sheaths were partially polarized.

The axonal spherical bodies observed in our study seem to represent lesions gradually formed due to glycoprotein denaturation over long periods of time and to be associated with leprosy-caused thickening of the perineurium of peripheral nerves.

RÉSUMÉ

Des corps sphériques, mesurant environ 10 µm de diamètre, qui n’ont pas encore été rapportés, furent trouvés dans les axones des nerfs périphériques prélevés à l’autopsie de patients lépreux.

Ces corps furent retrouvés dans les faisceaux de tous les nerfs périphériques des extrémités examinées (nerfs médian, radial, ulnaire, péroné et sciatique). Leur incidence n’était pas liée au type de lépre. La zone immédiatement en dessous d’un périneurium épaissi, un caractère associé à la lépre, était fréquemment riche de ces corps sphériques.

Lorsque observés au microscope électronique à transmission, ces corps sphériques montraient fréquemment une structure lamellaire, bien que certains étaient amorphes. Aucune structure ressemblant à une organelle ne fut décelée dans ces corps.

L’observation par une technique de concaténation a révélé une structure clairement lamellaire dans la grande majorité des corps sphériques. Ces corps et les manchons myéliniques environnants n’étaient que partiellement polarisés.

Ces corps sphériques des axones, observés dans notre étude, semblent représenter des lésions progressives à long terme de dénaturation des glycoprotéines et être associés aux épaissements du périneurium des nerfs périphériques causés par la lépre.

RESUMEN

Se observaron cuerpos esféricos de aproximadamente 10 µm de diámetro en los axones de especímenes de nervios periféricos colectados durante el examen post-mortem de pacientes con lepra. Estos cuerpos esféricos, que no se habían descrito antes, se encontraron en los fascículos de todos los nervios de las extremidades examinados incluyendo los nervios mediano, radial, ulnar, peronal y ciático. Su incidencia no estuvo relacionada con el tipo de
During our 40 years of experience of post-mortem examinations of leprosy patients, we have detected spherical bodies in the peripheral nerve axons of these patients. Many reports on peripheral nerve lesions observed in leprosy patients have been based on electron microscopy examinations. These reports have often mentioned the presence of *Mycobacterium leprae* in the axons, but none of them have reported the presence of axonal spherical bodies. For the study presented here, axonal spherical bodies were observed under light and electron microscopes at magnifications up to ×1000.

We used a new method known as merge technique (1), which allows for simultaneous viewing of an object under both a polarized microscope (PM) and a differential interference contrast microscope (DIC) within a common visual field.

**MATERIALS AND METHODS**

**Subjects from which specimens were obtained.** Peripheral nerve specimens collected from 6 cadavers during post-mortem examination at the “Oku Komyoen” National Sanatorium were used for this study. One of the specimens was relatively old (collected in 1983), but the other specimens were collected fairly recently (three in 1995 and two in 1996). The specimens were collected from 6 cases whose history follows. In all cases, leprosy-related peripheral nerve disturbance was observed.

**Case 1.** An 89-year-old male with lepromatous leprosy, who was diagnosed at age 17 with Hansen’s disease, and was admitted to the sanatorium when he was 27. At age 89, he was hospitalized because of mild motor paralysis of the extremities. During his hospitalization, he developed fever and increased sputum, leading to death from dyspnea in 1983.

**Case 2.** A 76-year-old female with tuberculoid leprosy, who was diagnosed at age 26 with Hansen’s disease and admitted to the sanatorium when she was 27. At age 71, she was diagnosed as having hepatocellular carcinoma during treatment for hepatitis C, and in 1995, she died of rupture of the cancer-affected liver.

**Case 3.** An 89-year-old male with lepromatous leprosy, who was diagnosed with Hansen’s disease when he was 25, and was admitted to the sanatorium 5 yrs later. At age 81, he was hospitalized because of anorexia, and 3 yrs later in 1995, he died of exacerbation of pneumonia.

**Case 4.** A 64-year-old male with lepromatous leprosy, whose diagnosis of Hansen’s disease was established when he was 14. The next year he was admitted to the sanatorium. At age 62, he was diagnosed with prostate cancer and began hormone therapy. When he was 64 years old, the cancer had metastasized to the stomach, and he died of deterioration of his general condition in 1995.

**Case 5.** A 79-year-old male with lepromatous leprosy, who was diagnosed as having Hansen’s disease when he was 24, was admitted to the sanatorium at age 27. He was diagnosed with a urinary bladder tumor when he was 77 years old and underwent several sessions of transurethral tumor resection. Two years later, when he was 79, he underwent a total cystectomy, but postoperatively developed metastasis of the cancer to the lumbar vertebrae, liver, subcutaneous tissue, and other areas. He died of cancer in the same year in 1996.

**Case 6.** An 81-year-old male with tuberculoid leprosy was diagnosed at age 21 as having Hansen’s disease and admitted to the sanatorium at age 26. When he was 81 years old, he developed chest pain and hy-
drothorax associated with thoracic aortic aneurysm, and his condition was com-
plicated by DIC, leading to his death in 1996.

Preparation of specimens. All speci-
mens of the peripheral nerves were fixed in
10% formalin. The specimens collected in
1995 and 1996 were 10 cm long were cut
into sections of about 2 cm. They were sub-
jected to HE, PAS, Bodian, Luxol fast blue,
amyloid and acid-fast bacterium staining.
Formalin-fixed specimens that were found
to contain spherical bodies were subjected
to PCR assay to determine the relationship
of the bodies to Mycobacterium leprae. The
specimens were also subjected to merge ob-
servation (allowing simultaneous observa-
tion under both PM and DIC in a common
visual field) and electron microscopy.

Merge technique. Non-stained, deparaffi-
inized specimens, 5–7 µm thick, were
mounted on acryl-based material. The speci-
men can be observed simultaneously in a
bright visual field under a biomicroscope
(BX51, Olympus), a DIC (Olympus) and a
PM (Olympus), without the need to move
the stage of any of the microscopes. Micro-
scopes capable of magnification up to
×1000 were used. Because DIC and PM
(two systems with different properties)
share the same polarizing filter (polarizer or
analyzer), switching from the DIC to the
PM image and vice versa can be done
rapidly, without any distortion in the visual
field. At room temperature and under ordi-
nary fluorescent light, polarized images
were obtained in the FL and differential-
interference images in the BF mode with a
digital camera (DP-50, Olympus). The im-
ages thus taken were subjected to image
analysis using the Photoshop (Adobe) soft-
ware package. During image analysis, pol-
arizing images were overlapped with
differential-interference images of the same

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**Fig. 1.** Case 1. Several axonal spherical bodies are visible, some of them granular. These bodies were often
detected immediately below the thickened perineurium (arrow). (×400, PAS).

**Fig. 2.** Case 1. Two axonal spherical bodies are visible, one lamellar and the other amorphous. The
surrounding myelin sheaths are degenerative. (×1000, PAS).
visual field and with the same number of pixels.

Polarizing images can be used to check for polarizing materials, while differential-interference images provide a view of the entire photographed area. Merged images then make it possible to determine the exact location of the polarizing material.

RESULTS

Spherical bodies found within peripheral nerve axons under a light microscope. The diameter of most of the axonal spherical bodies was about 10 µm, with one section usually containing 1 or 2 spherical bodies. It was rare for 3 or more bodies to be observed in one section. The bodies were visible in HE-stained sections, but more so in PAS-stained sections (Fig. 1). They were not stained by acid-fast staining and often had a lamellar structure, although some did not show any specific structure. The bodies were often spherical or oval, and some of them were composed of several small granules of irregular size.

A narrow area characterized by irregular swelling was often seen around the spherical bodies within the axons (Fig. 2). The lesions were more frequently seen immediately below the perineurium, and they were detected in all peripheral nerves examined (median, radial, ulnar, femoral, perinea and sciatic nerves). In Bodian-stained sections, spherical bodies are in the axons and the silver particles are in the center (like a core) (Fig. 3). In Luxol fast blue-stained sections, the spherical bodies did not stain at all (Fig. 4). Their incidence did not correlate with the type of leprosy. These spherical bodies showed no chromatic response to amyloid staining, and, when assayed by PCR, were found to have no relationship to Mycobacterium leprae (data not shown).

Findings from transmission electron microscopy. The spherical bodies which appeared to have no specific structure under the light microscope were also amorphous when observed under the electron microscope. The bodies with a lamellar structure under the light microscope, were found under the electron microscope to contain fine powder-like materials with a high electron density in their center (like a core), and show a lamellar structure composed of rings with different electron densities (Fig. 5). The myelin sheaths surrounding the spherical bodies were of irregular thickness (Fig. 6).

Observation with the merge technique. A few of the spherical bodies showed partial polarization, while small areas within
the myelin sheaths surrounding them were sometimes polarized (Figs. 7, 8). Most spherical bodies had clearly lamellar structure. A number of minute granular or rod-shaped polarized inclusions were visible in the histopathology specimens. The polarization disappeared after treatment of the sections with an alkaline solution.

**DISCUSSION**

Although a number of reports (3) have been published concerning peripheral nerve lesions associated with Hansen’s disease, none of them have dealt with axonal spherical bodies. A search of previous findings resembling the spherical bodies we detected in peripheral nerve axons, revealed that the

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**Fig. 4.** Case 1. A spherical body does not stain at all. (×1000, Luxol fast-blue).

**Fig. 5.** Case 1. Core-like material is visible in the center of the axonal spherical body showing a lamellar structure. (electron micrograph).

**Fig. 6.** Case 1. An amorphous spherical body is visible within the axon. The surrounding myelin sheaths vary in thickness. (electron micrograph).
ballooning and fragmentation of axons (Bodian stain) shown in Fig. 3 of the report “Pathology of Peripheral Nerve Lesion in Lepromatous Leprosy,” published in 1971 by Job (4), resembled our spherical bodies. The spherical body and “spheroid” which appears pathologically at the time of amyotrophic lateral sclerosis differ from each other in the size and the location.

While tissue specimens, sealed in fixation bottles, were available from not only the six cases included in this study but also from some earlier cases, they had been collected in various ways because post-mortem examinations had been performed by several different pathologists. These earlier specimens were therefore not used for our study because it would be difficult to perform light microscopic observation of all nerve specimens under identical conditions. We do not think that the spherical bodies result from the post mortem change, because the spherical bodies can be seen in both new and old specimens. The patients’ illnesses had progressed over more than 10 yrs. We did special stains (Bodian and Luxol fast-blue stain) for the relation between these spherical bodies and the axon or myelin substance. But this special stains showed no relation between the spherical bodies and the axon or myelin substance.

Under the light microscope, a narrow area characterized by irregular swelling was often observed around the spherical body within the axon. This area seemed to represent a precursor lesion. Among the other areas of the axon, without swelling, the spherical body was often seen in the area where the peripheral perineurium had thickened and become abnormally hard. In this connection, it is interesting that Kimura, et al. (2) reported that the perineurium of the peripheral nerves appears to be a target of *Mycobacterium leprae*.

When observed under the transmission electron microscope, the spherical lesions often showed a lamellar structure, although some lesions had no specific structure. The lesions contained nothing resembling organelles, nor showed any chromatic response to amyloid staining, indicating that the lesion did not represent amyloid degeneration. The fine powder-like material with a high electron density observed in the central area and resembling a core, differed in hardness from the other areas. This material made it difficult to slice the specimens into sections suitable for electron microscopy.

![Fig. 7. Case 2. (left: DIC, middle: merge technique, right: PM) Two partially polarized axonal spherical bodies are visible, with the degenerative surrounding myelin sheaths showing some polarized spots. Degenerative connective tissue also shows wave-formed polarization. Polarization of distribution is more evident on the image taken with the merge technique.](image)

![Fig. 8. Case 2. (left: DIC, middle: merge technique, right: PM) A group of axonal spherical bodies with polarization mostly absent, except for some polarized spots.](image)
When observed by the merge technique, the area around the spherical bodies and the myelin sheaths surrounding them was partially polarized in some specimens, although infrequently. Most of the spherical bodies had a clearly lamellar structure, which is consistent with the findings from HE and PAS staining and electron microscopy.

The axonal spherical bodies were clearly PAS-positive, and calcification-like deposits were occasionally seen in their center. These findings, combined with that of the lamellar structure, make it appear likely that the bodies were gradually formed over long periods of time. These bodies were seen in cases of both lepromatous and tuberculoid leprosy. In conclusion, the axonal spherical bodies observed in the study presented here seem to represent lesions gradually formed due to glycoprotein denaturation over long periods of time and are associated with leprosy-caused thickening of the perineurium of peripheral nerves.

Acknowledgment. The authors are indebted to Mr. K. Fukuike (technologist) for his help in preparing the pathology specimens and to Mr. T. Yamada (Olympus Plaza Osaka) for his help with using the merge technique for taking the DIC and PM pictures. The authors also wish to thank Prof. C. Ide (Department of Anatomy, Kyoto University) for the electron micrographs he prepared for this study and to Dr. K. Saeki (National Sanatorium Oshima Seishoen) for the PCR assay of formalin-fixed peripheral nerve specimens he conducted for this study.

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Proper classification of a disease is one of the fundamental tools of modern medicine in selecting treatment, evaluating prognosis, measuring overall progress, and furthering the understanding of that disease. Classification is an essential tool in our approach to a disease, much as a good map is necessary tool for developing and navigating a country. The development of a comprehensive, practical classification system for leprosy may rate as one of the most important accomplishments in the extraordinary progress against this disease in the 20th century. While the more obvious, essential accomplishment was the discovery of effective anti-microbial agents to treat this infection, the availability of effective treatment does not, by itself, guarantee success against a disease. The value of classification is not only historical; continued application of the best classification system is essential in the efforts to better understand a disease and to clearly and intelligently develop a strategy to combat it and, ideally, prevent it.

The struggles against leukemia and lymphoma provide a useful example of the continuing value of classification. Several effective agents were available for these malignancies long before many of the current successes in treatment. Greater success has been brought about in part by a better understanding of how to use these agents in combination, but also—and very importantly—by better classification systems that enable physicians to know which types of leukemia or lymphoma will respond to particular medicines or combinations. Recognition of this has stimulated continued, vigorous research to refine the methods and concepts of classification of many malignancies. Such studies enhance the understanding of these diseases, with implications not only for treatment but also for early detection and prevention. Today, no cancer researcher would consider conducting a study, or publishing results of a study, using a primitive or technically outmoded classification system. And no professional journal would accept such a report.

Yet, in current leprosy research, a disturbing trend is to do exactly that: to abandon the best classification system (one that uses clinical assessment plus histopathologic examination of a skin biopsy), and choose instead to group patients into only 2 groups, multibacillary (MB) or paucibacillary (PB), according to their bacterial load, or to disregard the bacilli altogether and classify according to the number of skin lesions on a patient’s body. This last approach, which disregards the bacteria entirely, seems highly ironic for research on an infectious disease. These approaches are technically inferior ones that assume, and accept as satisfactory, a higher degree of inaccuracy than is readily available with standard technology. Such over-simplification of this complex host-pathogen relationship is unfortunate and unacceptable. It is as if some have grown intellectually weary of
trying to understand the full-color immunopathologic spectrum, and have decided to settle for a black and white outline.

Why is this done? Two related reasons are generally given—cost and expertise. The MB/PB categorization was promulgated by the World Health Organization in the global campaign to eliminate leprosy as a public health problem. For some treatment and control programs, where access to expertise and other resources is severely limited, the use of a simplified system of classification is reasonable, just as paramedical workers deliver medical care where there are no doctors. It has become too easy, however, to use this as an excuse to justify a non-critical acceptance of oversimplification that does a disservice to our basic research endeavors.

Research always requires substantial expertise and is inherently costly, and is nearly always conducted with specifically allocated research funds. Nevertheless, we have watched with dismay as some investigators and collaborative groups apply sophisticated molecular and immunologic techniques to specimens from patients who are classified only as MB or PB, or are classified only according to the number of skin lesions. Some of these manuscripts arrive at our office, and some are published in other journals. The multiple authorship and acknowledgments of support in most of these papers clearly indicate that financial resources and sophisticated expertise have been brought to bear, and funds have been allocated for expensive instruments and reagents. Experienced clinical leprologists are virtually always involved in these studies, implying an availability of sufficient resources, and it is not acceptable that they do not take the effort also to obtain skin biopsies and have them examined by an experienced professional.

In some instances the pressure to publish quickly appears to play a role. The Ridley-Jopling classification system (1) divides patients into five groups, whereas MB/PB schemes divide into only two groups. It is much easier (and faster) to obtain enough patients for a 2-group protocol than for one with 4 or 5 groups. But is this better? Is knowledge really advanced by such a simplification? We are very skeptical. No self-respecting academic research advisor will accept such an excuse from a student, and the research community should not make an exception and settle for this with respect to research on leprosy.

Any classification system must be applied thoughtfully, and in some circumstances a simpler system truly will suffice. For example, in epidemiologic or implementation field studies, simplified classification may be justified.

The diversity inherent in the immunologic spectrum of leprosy may not be reflected in all biological parameters we set out to measure. In some instances, the results may reveal that patients fall into only 2 or 3 groups. To discover this is not a failure, nor is it wasted effort. Once such findings are established, a 2- or 3-part classification scheme for that parameter is acceptable. But if the hypothesis is not first evaluated against the full spectrum, we will not know if there were more than 2 or 3 groups. The burden remains on the investigators, however, to explain why better classification was impractical and why a simplified system is actually acceptable in testing their hypothesis. If we do not look, we will not know conditions as they truly exist, and we may thus overlook important connections and implications.

Researchers in leprosy have before them, at all times, one of the great immunological models in nature. An essential part of the foundation of our understanding of leprosy is the recognition that—clinically, histologically and immunologically—polar lepromatous (LL) differs from borderline lepromatous (BL), and borderline tuberculoid (BT) differs from polar tuberculoid leprosy (TT). From their first publication in the mid-1960’s, the soundness of the theoretical basis for this classification system (2), and the description of practical, straightforward criteria to accomplish such classification anywhere in the world (3), were hailed as major accomplishments by workers within and beyond the field of leprosy. Both the theory and the practical criteria recognize the natural diversity of the immune response in leprosy that has challenged immunology for nearly half a century. A more complete understanding of the basis for this diversity and its underlying mechanisms will most probably be required before this disease can be eliminated (i.e., before a highly effective vaccine can be developed).
The questions posed by this complex immunopathologic spectrum have perplexed more than a few great minds who have attempted to tackle them. Although support for leprosy research has declined, it seems a grave mistake for those of us who continue to work on leprosy to surrender one of our best scientific assets—a practical and theoretically sound classification system for leprosy. Oversimplification fosters the illusion that this disease is simpler than it appears, and easy to understand (or eliminate). Infection with *Mycobacterium leprae* elicits the full range of human immunologic responses; this is a natural phenomenon and, like the metastasis of cancer, it will not go away if ignored, but will be ignored at our peril.

Today, although the prevalence of leprosy has declined worldwide, the number of new cases diagnosed annually has not. This paradox raises new, important, and interesting questions. Answering these and the other still unanswered questions about leprosy will require application of the best scientific methods available. It is common knowledge that funds for leprosy research are in much shorter supply than they were a few years ago, and that fewer individuals are engaged in leprosy research. This, however, is not an excuse for us to be less rigorous. To do so would be a travesty to the hundreds of thousands of patients still diagnosed every year, to those with lasting disabilities from this disease, and to all of those who have gone before us, who did not shrink from a rigorous attempt to understand the complexity of leprosy even though they worked without many of the technical advantages we have today.

—DMS

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Genomic strategy to identify the chromosome 6 leprosy susceptibility gene. Although the localization of the leprosy susceptibility locus to chromosome 6q25 was an enormous breakthrough, the task of identifying the responsible gene still presented major difficulties, since the target region delineated by the two markers D6S4155 and D6S1277 covered approximately 6.4 million nucleotides that encoded 31 known genes (2). Since the region was too large to attempt straightforward comparative DNA sequencing, another strategy was needed to dissect out the gene. Thus a panel of 64 single nucleotide polymorphisms (SNPs) was selected that tagged each of the 31 genes in the core interval by at least one SNP marker (3). The SNP markers used for the Mira, et al. study were selected either directly from the human SNP database or obtained by comparative sequencing of unrelated Vietnamese individuals. Next, DNA samples were isolated from all members of 197 Vietnamese families composed of two healthy parents and one leprosy-affected child, so called “simplex” families. These DNAs were genotyped for the 64 SNPs, and systematic analysis of association with leprosy disease was performed for each SNP marker (“association scan”). Importantly, the phenotype employed was leprosy disease independent of specific clinical paucibacillary or multibacillary forms (PB or MB) of the disease. The association scan showed that SNPs with strongest association were located in an 80 kilobase fragment that overlapped the promoter (regulatory region) of the PARK2 (parkin) and PACRG (parkin co-regulated gene) genes. A higher density screening with a further 81 SNPs in the 80 kilobase segment further confirmed the regulatory promoter region and a total of 17 SNP markers were found associated with leprosy disease. By conducting a multivariate analysis, it could be shown that only 2 of the 17 SNPs captured the entire association between the 80 kilobase fragment and leprosy disease. Most importantly, the findings in the Vietnamese families were confirmed in patients from a second leprosy endemic country (3). A subsequent analysis found that the SNP markers associated with leprosy in Vietnam were also associated with leprosy susceptibility in 975 unrelated individuals from Brazil. In both populations, the risk alleles are associated with leprosy per se, meaning that persons carrying the risk alleles would be equally likely to develop PB as MB. Therefore, the cause of susceptibility is likely to involve an early, common cellular pathway used by the M. leprae bacillus. While the study conclusively implicates PARK2 and/or PACRG in leprosy pathogenesis, the question if any of the leprosy-associated SNPs is directly and causally involved in leprosy susceptibility remains unanswered.

PARK2 and PACRG genes reveal the function of a novel cellular pathway in susceptibility to leprosy infection. The possible identity of a “leprosy per se” pathway was revealed through knowledge of the function of the PARK2 and PACRG genes (1–3). Mutations in PARK2 are responsible for familial early-onset Parkinson disease (PD), which represents approximately 3% of all PD cases. The mutations in PARK2 in PD are not found in leprosy patients. The
PARK2 (parkin) gene product is an ubiquitin-protein ligase, which activates deposition of certain proteins such as alpha-synuclein in so-called intracellular Lewy bodies. The lack of ubiquitin ligase activity in patients with PARK2 mutations causes protein accumulation and neurodegeneration. PACRG appears to be involved in the transport of polyubiquitylated proteins to the proteasome. Overall, PD is a complex disease with both genetic and environmental factors, and it has been suggested that infections may trigger its onset. However, neither PARK2 or PACRG genes have yet been associated with susceptibility to any infectious disease other than leprosy. One important aspect to determine then is whether the PARK2 or PACRG associations are found only in leprosy, or are associated with other mycobacterial diseases, such as tuberculosis, and, if the polymorphisms associated with leprosy also predict risk of PD.

In vitro experiments to determine the function of the leprosy susceptibility gene. The primary focus now should be to establish a connection between the risk alleles for leprosy susceptibility, the ubiquitin proteolysis pathway and the course of M. leprae infection and growth. There are many unknowns to this next phase of experimentation. For example, it is not known whether the leprosy susceptibility alleles would up or down regulate the PARK2 or PACRG encoded proteins, or affect the cellular ubiquitin pathway. In the context of further functional studies, it is revealing that both PARK2 and PACRG are expressed by Schwann cells and monocyte-derived macrophages (3). Nevertheless, testing of the risk alleles in patients will have to await a reliable functional assay for biological activity of M. leprae. In parallel, it is also hoped that the analysis of M. leprae and the parkin genes will yield information relevant to the neurological aspects of Parkinson’s disease.

CONCLUSION

The clinical spectrum of leprosy has long been recognized as an immunological model in which various aspects of human T cell subset and cytokine function can be characterized (2). It is fitting that leprosy has proven once again to be a model for molecular genomics, by providing the first infectious disease locus isolated by positional cloning. Since this success is largely rooted in the framework provided by the Human Genome Project, the identification of this novel and entirely unexpected leprosy susceptibility locus also provides a good example that recent advances in genomics can be used for the study of diseases primarily prevalent in resource-poor countries. Taken together, the papers by Mira, et al. provide a general framework for the genetic analysis of complex infectious diseases. Above all, it is hoped that the novel link of leprosy susceptibility to the ubiquitin proteolysis pathway will yield some insight to the transmission of leprosy, a disease which has so far evaded eradication despite many years of effective drugs and case finding (6).

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REFERENCES


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Neuropathic Pain in Leprosy

ABSTRACT

Neuropathic pain appears to be much more common in leprosy than has been generally appreciated. Emphasis in leprosy control programs has been on the distribution of multi-drug therapy, on early and better detection, and on the prevention of disability related to anesthetic limbs. Most have thus been inattentive to the problem of neuropathic pain in leprosy patients. Neuropathic pain does not respond to the usual analgesics employed for reactions, for example, and so it is important that those treating leprosy patients give this problem the special attention it requires, both in diagnosis and in treatment.

RÉSUMÉ

Les douleurs neurogènes de la lèpre pourraient bien être beaucoup plus fréquentes que ce qui a été considéré auparavant. L’effort des programmes de contrôle de la lèpre a été orienté vers la distribution de la polychimiothérapie, une détection meilleure et plus précoce et dans la prévention des handicaps associés à des membres anesthésiés. La plupart des programmes ont ainsi apporté peu d’attention au problème des douleurs neurogènes chez les patients hanséniens. Les douleurs neurogènes ne répondent pas aux analgésiques usuels employés pour traiter par exemple les réactions et il est donc important que les personnes engagées dans le traitement des patients hanséniens considèrent particulièrement cet aspect, tant en ce qui concerne le diagnostic que le traitement.

RESUMEN

El dolor neuropático en la lepra parece ser mucho más común de lo que usualmente se considera. Los programas de control de la lepra han hecho mucho énfasis en la distribución de drogas para la poliquimioterapia, en la temprana y mejor detección de la enfermedad, y en la prevención de las incapacidades relacionadas con los miembros anestésicos. Muchos programas han puesto poca atención al problema del dolor neuropático en los pacientes con lepra. El dolor neuropático no responde a los analgésicos usualmente empleados en las reacciones de la lepra, por ejemplo. Por esto, es muy importante que los encargados del tratamiento de los pacientes con lepra den a este problema la atención especial que requiere, tanto en el diagnóstico como en el tratamiento.

Stump, et al.’s paper, “Neuropathic pain in Leprosy patients,” published in this issue of the JOURNAL, is a timely and important contribution to the evaluation and management of leprosy sufferers. In the wider medical world the management of chronic pain is developing as a specialty in its own right complete with journals and international conferences devoted to the subject. It is an interesting coincidence that a review article on the same topic has just been published in the most recent edition of Leprosy Review (1).

In the leprosy world, we have been slow to catch on to the existence of chronic neuropathic pain occurring in leprosy patients. Hastings’ textbook Leprosy (1995) does not mention it at all, and there have been remarkably few papers published on the subject in the world leprosy literature. Yet Stump and his colleagues report that 56% of the 358 patients assessed for neuropathic pain in his study either had experienced or were experiencing episodes of pain of sufficient intensity to interfere with activities of daily life or sleep. The statistics they adduce are in line with findings from the few other studies that have been carried out amongst leprosy sufferers. How could we have missed it for so long?

There are several overlapping answers to that question. For several years most programs, NGO and Government alike, have simply been extremely busy and focussed on case finding and multi-drug therapy (MDT) administration. This has been extraordinarily successful in reducing prevalence and clearing the backlog of patients in the community. In many places the heat has

1 Received for publication on 23 March 2004. Accepted for publication on 14 April 2004.
now come out of that approach and perhaps there is a little more space to reflect on what our patients—including “cured” patients—are actually experiencing. Another important focus in leprosy programs and in research of the last decade or so has been the detection and management nerve function impairment (NFI) and the prevention of disability. Both of these foci—detection and treatment, and prevention of disability—have had anaesthesia at the hub, since it is the absence of sensation that leads both to the diagnosis of leprosy (and therefore to treatment), and to the development of the most damaging disabilities and consequent handicap and stigma. We have been so attuned to painlessness that we have missed the fact that a very significant proportion of our leprosy sufferers experience pain as part of their dis-ease. Furthermore, they may continue to suffer long after they have been declared “cured” and are lost to follow-up. That we should have been so deaf and blind to this most basic of complaints—pain—is extraordinary.

As already alluded to, there has been a paucity of studies into neuropathic pain carried out amongst leprosy sufferers and very few references to it at all in the world literature. There is a clear need for more research into this subject and for the findings to be applied as rapidly as possible. However, much is known already about the diagnosis and management of neuropathic pain in general that could easily be applied now. If it is as common as Stump et al. suggests—and it probably is—then we should get on with it now.

If we are to begin to help leprosy sufferers with chronic neuropathic pain then as a first step we must ask them about it. It should not be left to experts in research centers to ask the questions; it should become part of the routine history taking of every paramedical worker. Before that can happen, training institutions must incorporate this message. Leprosy workers need to understand the difference between nociceptive and neuropathic pain, and Stump, et al. rightly draw attention to this. Crucially, it should be understood that neuropathic pain will not respond to simple analgesia, but rather to different drugs such as tricyclics and anticonvulsants. Then, the treatment of neuropathic pain must become mainline.

Perhaps a good word to use here is demystify. Neuropathic pain is regarded by some doctors as a little technical, rarefied even. The subject needs to be demystified; it needs to make the jump to become the regular.

Perhaps the situation we are in is akin to the situation that existed a decade or so ago, before the widespread use of corticosteroids at “field level” to treat acute NFI and reactions. We knew how to measure NFI, and we had an effective drug, prednisolone, but it took a paradigm shift in thought and practice for this technology to be widely and simply applied so that the maximum number of people could benefit. In the same way, it is known how to diagnose neuropathic pain (and it is not difficult), and at least one very cheap and effective drug is available (amitriptyline). A widespread application of this knowledge down to the grassroots level could be of considerable benefit to a large number of people.

The current cut-and-dried WHO recommendations for the treatment of leprosy focuses very much on bacteriological cure with discharge after relatively short courses of treatment. It is well known that this largely ignores the existence of new nerve damage after release from treatment, but to date there has been very little appreciation of the way that this ignores the presence of neuropathic pain among “cured” leprosy sufferers, as Stump et al. points out in his conclusions. Indeed, the prevalence of neuropathic pain he found is actually higher than that often quoted for NFI amongst leprosy patients.

In summary, Stump’s paper both documents and highlights the existence of a common and significant problem amongst leprosy patients, one that has been remarkably overlooked. There is a need to demystify the diagnosis and treatment of neuropathic pain and to develop simple strategies that will enable the widespread application of simple and effective techniques for its management.

—Richard Croft

REFERENCE

TO THE EDITOR:

According to World Health Organization (WHO) recommendations: “five, or lesser number of lesions” of leprosy should be treated as paucibacillary (PB) leprosy and should be given 6 months treatment with rifampicin monthly and dapsone daily (5, 6). In this type of simplified classification, the size of the patches are not considered. However, we feel that size of the patch should be considered on deciding whether to treat a case as PB with two drugs for 6 months, or as multibacillary (MB) leprosy with three drugs for a year. Categorizing leprosy as PB or MB is particularly important in areas where treatment is commenced without any bacteriological and histopathological confirmation. Even in the time honored Ridley-Jopling Classification and its modifications, large patches of leprosy are considered as a feature, more commonly found in border-line, borderline tuberculoid, or subpolar lepromatous leprosy (2, 4), giving due consideration to the size of the lesions.

Histopathologically, in tuberculoid leprosy there are tubercles composed of epithelioid cells. This is due to the process of destruction of lepra bacilli by histiocytes (3). The granulomatous reaction thus produced is the result of a combination of the presence of bacilli and the host response. Considering that sensory impairment and pathological hypopigmentation in leprosy are due to this host response by the body in the fight against the leprosy bacilli, it is likely that, the larger the lesions of leprosy, the higher the number of bacilli that cause the pathology. A granuloma which originates due to one or more bacilli in a given area can only cause a very limited spread of its effects, e.g., focal sensory loss in the affected area. The fact that inoculation of atypical mycobacteria causes a granuloma in the immediate vicinity of the inoculation, and that it spreads very slowly, suggests that proliferation of bacteria are necessary to cause a larger lesion. This also means that if there is no proliferation of bacilli in tuberculoid leprosy, there can not be evolution of a small patch, to become a large patch. This concept is further supported by the fact that even in a Type I leprosy reaction, there is no real lateral spread of a leprosy lesion, though the existing lesions temporarily become inflamed. This suggests that a pure immunological response without an increase in bacilli is unlikely to cause a lesion to spread peripherally, to produce a large hypopigmented patch. However, it is known that lowering of one’s cell mediated immunity is important in promoting the spread of leprosy lesions. In this situation, the patient’s ability to destroy the multiplying lepra bacilli is impaired, allowing the lesion(s) to enlarge; as in the case of a tuberculoid leprosy (PB) lesion or lesions in an untreated patient, evolving towards the “lepromatous pole” (MB) over several years.

Unless many individual cutaneous nerve

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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.

Should Large Lesions of Leprosy Be Considered As “Multibacillary” for Treatment Purposes Even If the Total Number of Lesions Is Less Than Five?1
fibers are affected by separate bacilli, there can not be sensory impairment in a large area, involving the total area of the hypopigmented macule. This is different to distal sensory impairment due to a nerve trunk involvement, for example, sensory impairment along ulnar nerve distribution. Furthermore, in monitoring tuberculoid or borderline tuberculoid leprosy, peripheral extension of a lesion is considered to be a feature of failure of treatment or relapse.

Although relapses of leprosy after treatment are reportedly uncommon, many authorities feel that they may be underestimated (1). If an MB case is misdiagnosed and treated with dapsone daily and rifampicin monthly as a PB case, that patient receives only 6 doses of the bactericidal drug rifampicin before stopping the treatment. This would be totally inadequate. Some authorities even believe that MB treatment should be continued for 24 months rather than the WHO recommended 12 months (1). In countries where leprosy is still highly prevalent, follow-up after discharge from active treatment is unsatisfactory. Therefore many relapses or suboptimal treatments may go unnoticed for many years.

Considering the above facts, we feel that where a large patch (more than 10 cm in diameter) of leprosy is present, irrespective of the size of the other lesion or lesions, the patient should be treated as MB and given treatment at least for 12 months. Just as “5 or less leprosy macules are considered as PB” (as recommended by the WHO) is an arbitrary limit, “the dimensions of a lesion” is also an arbitrary measurement, for places where microbiological and histopathological services are unavailable. It should also be emphasized that counting lesions can be erroneous if the whole body is not carefully checked by the healthcare worker. A person may have 5 easily visible lesions, but there may be another small lesion or lesions in unsuspected places such as nasal cleft, a toe, or an elbow posteriorly. In such a situation, a patient would receive only PB treatment. However, it is highly unlikely that a large patch (>10 cm) of leprosy would go undetected by the patient or the clinician or the health care worker.

In our experience with cases of leprosy in the last two decades (mostly when working in Sri Lanka) we have encountered several instances where MB cases had been categorized and treated as PB, by others, especially by public health workers, due to their strict categorization according to the “number of patches.” These cases were subsequently given the MB treatment regimen. In hospital settings, facilities for biopsy and smears with microbiological evaluation are available and clinicians do not go by the number of patches alone for treatment.

Long term follow-up of patients with large macules of leprosy treated with the standard WHO treatment regimens would be necessary to ascertain whether relapse rate is higher in this group of patients. Personal experience suggests this group has more relapses or non responders.

A consensus on the duration and type of treatment for large macules of leprosy would be desirable for places where histopathological and microbiological facilities are not available. It appears prudent to treat such cases with MB treatment regimen.

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REFERENCES

TO THE EDITOR:

It is often difficult to diagnose early leprosy by clinical criteria alone. Frequently, the patients’ skin smears are negative, clinical findings inconclusive, and history unreliable and subjective. In many such cases, routine histopathology may also be non-specific as *Mycobacterium leprae* cannot be demonstrated in the tissues of many early lesions. Therefore, the demonstration of *M. leprae*, or any of its components in a tissue biopsy, is crucial and important to reach a definitive diagnosis.

Polymerase chain reaction (PCR) amplification has been successfully used to detect extremely low numbers of *M. leprae* in fresh unfixed human skin biopsy specimens, providing powerful direct and unequivocal tests for diagnosis of leprosy (1, 2, 3, 4, 5, 7, 8, 10, 11). However, these studies required the specimens to be processed and analyzed promptly, in addition to being properly stored and transported. Adoption of PCR technology for detecting *M. leprae* and/or its components in fixed tissues would give clinicians the option of examining biopsy specimens for the presence of *M. leprae*, which along with histology would help in arriving at a definite diagnosis. This PCR technology would be of great help to arrive at a quick and conclusive diagnosis, identify and treat early cases of leprosy, to differentiate leprosy from non-leprosy cases and also for epidemiological purposes.

While earlier reports have demonstrated that buffered formalin was good for both histology and PCR detection, formalin fixation of skin biopsy specimens for longer than 24 hr has been reported to have an inhibitory effect on PCR amplification (2).

Therefore, we here report the development of improved protocol for PCR diagnosis of *M. leprae* from formalin-fixed specimens and report the results of a blind study using a large number of biopsies obtained from both paucibacillary and multibacillary leprosy patients.

**MATERIALS AND METHODS**

After obtaining informed consent and using aseptic precautions and techniques such as cleaning of skin with iodine solution and alcohol, 5 mm punch biopsies were taken from 78 patients of Indeterminate, polar tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline-lepromatous (BL), or polar lepromatous (LL) leprosy cases from the Ghatampur field area of Kanpur district, India. For controls, biopsies from 12 cases of other dermatological conditions (Pityriasis alba, Tinea versicolor, and Vitiligo) from the same population were also taken and analyzed using the same protocol. These biopsies were fixed and transported in buffered formalin and processed in a blind manner after 5 to 7 days. The scientists performing the gene amplification assay were unaware of the diagnosis of the case, and that biopsies of other dermatological cases were also included in the study. Each biopsy was divided into two parts: one part was used for histology and the other for the gene amplification assay.

Initially, the biopsies were kept in 15 ml of sterile distilled water for 8 hrs, which had been determined to be optimal after testing with various time periods. The biopsies were then aseptically homogenized in 1 ml of sterile T. E. buffer (Tris 10 mM, EDTA 0.1 mM, pH 8.0) using pestle and mortar in a bio-safety hood.

A technique based on the principle of a combined physiochemical approach, first freeze-boiling and then treatment with lysozyme-proteinase K (7) was used for extraction of DNA. Briefly, the homogenates in T. E. buffer were frozen, thawed, and

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then enzymatically treated at 37°C, first with lysozyme (3 mg/ml) for 2 hr followed by proteinase K (250 ug/ml) treatment for one hour at 65°C (°). After de-proteinization with chloroform: isoamyl alcohol (24:1), DNA was precipitated with 0.6 volume of isopropanol. This was dissolved in T. E. buffer and stored at −20°C until further use. Primers and the gene amplification method as described by Hartskeerl, et al. (0) and as used by de Wit, et al. (1) and Singh, et al. (8) were followed for the amplification of 36kD gene of *M. leprae*. Primers were obtained commercially from Bioserve Biotechnologies (India) Ltd. In all cases, 35 cycles were used for the amplification and confirmation was done by the southern blot analysis with random digoxygenin (DIG) labeled amplified 36 kDa gene fragment of *M. leprae* as a probe. DIG labeling and detection was done by using a kit from Roche Diagnostics (Cat. No. 1093651).

**RESULTS**

The percentage positivity of biopsy samples for *M. leprae* by gene amplification is shown in The Table. It was observed that 48% of Indeterminate, 55% of TT/BT, and 83% of BB/BL were positive by gene amplification. The lone BB patient who was negative by this assay was smear-negative and had taken multi-drug therapy (MDT) for more than a year prior to the biopsy. None of the specimens of non-leprosy cases were positive by this method, demonstrating the specificity of the method.

For histologic investigations, tissue samples are mostly stored as formalin-fixed, paraffin-embedded blocks. Widening the applicability of amplification techniques to formalin-fixed blocks could improve the routine diagnosis of mycobacterial infections. This is particularly important in leprosy as *M. leprae* cannot be grown in any in vitro system. Definite diagnosis of early and suspicious cases of leprosy is required, so that the patients can be treated at an early stage to prevent development of deformities, rather than following them up closely and treating them when definite clinical signs appear. While large studies are necessary to gain more confidence, published information shows that with PCR technology, identification of *M. leprae* is sensitive, as well as and specific (1, 3, 5, 7, 8, 10).

<table>
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<th>No. tested</th>
<th>No. positive</th>
<th>% positivity</th>
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<td>Indeterminate</td>
<td>25</td>
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<td>48</td>
</tr>
<tr>
<td>TT/BT</td>
<td>47</td>
<td>26</td>
<td>55</td>
</tr>
<tr>
<td>BB/BL</td>
<td>6</td>
<td>5</td>
<td>83</td>
</tr>
<tr>
<td>Non leprosy cases</td>
<td>12</td>
<td>0</td>
<td>Nil</td>
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It would be ideal if the PCR could be performed on fixed specimens in which both the detection of *M. leprae* and/or its components, and correlation with the histopathology, can be made. Over the years, different fixatives have been tried for histology and DNA, RNA/DNA of the causative organisms. Fiallo, et al. (0) and deWit, et al. (1) have observed significant reduction in sensitivity of PCR for *M. leprae* DNA when Zenker’s fluids (mercuric chloride) fixatives are used. Although Carnoy-Lebrun’s fluid is recommended for fixation of tissues for optimal staining of glycogen and RNA, unfortunately this fixative has the potential for chemical modification, resulting in degradation and excessive depurination of the DNA due to the acidic nature of fixative. Ethanol was reported to be a good fixative for PCR analysis, but this causes excessive shrinkage of the tissues, and is not recommended if the same tissue is to be used for histology and PCR analysis. Neutral buffered formalin fixation has been reported to be satisfactory for subsequent PCR, but significant reduction in the level of PCR signals for *M. leprae* DNA was observed after fixation of 4 to 7 days (°). In the case of formalin-fixed, paraffin-embedded specimens of smear-positive tuberculosis, the highest sensitivity rates have been obtained by amplifying the highly repetitive IS 6110 insertion sequence, and the different primers tested showed a sensitivity ranging from 80% to 87%, whereas a lower positivity of 47% to 80% with primer targeting single copy gene was observed in same study (°).

In the present study, after fixation of biopsy specimens and processing these within 5 to 7 days for PCR, 83% positivity was observed in multibacillary BB/BL patients and 43% to 55% in paucibacillary
Singh, et al.: M. leprae-PCR on Fixed Biopsies

(Indeterminate and BT). This is similar to the positivity rates of 50% to 70% for smear-negative and about 100% in smear-positive cases observed with fresh frozen biopsy specimens in earlier studies \(^\text{5,7,8}\). Prior to this study it has been reported that formalin fixation of tissues for longer than 24 hrs is detrimental for PCR detection of \textit{M. leprae}. It appears that this study provides a major improvement in the ability to detect \textit{M. leprae} in tissues fixed in buffered formalin.

This improvement is most likely due to the soaking of the tissues for 8 hrs in 15 ml of sterile distilled water prior to homogenizing tissues, lysing and extraction of DNA. In the study of Fiallo, \textit{et al.}, tissues were soaked in HBSS solution for 30 minutes. In the present study, the tissues were collected as well as processed in buffered formalin which did not inhibit the PCR amplification, and the results are comparable to the earlier reports when PCR amplification was done in freshly biopsied samples obtained in the outpatient clinic of the Institute. It appears that the approach of collecting the specimens in buffered formalin will be very suitable for field situations and will have the added advantage of the same specimen being used for histology, probe application in solution, as well as for \textit{in situ} applications. This, however, is a pilot study and trends need to validated by similar studies on a large number of specimens using the same method by other workers.

Acknowledgment. Authors are thankful to all technical and secretarial colleagues for support in carrying out this study and preparation of this manuscript. Gift of reagents/plasticware by LEPRA (UK) is gratefully acknowledged. This study has been supported by the Department of Biotechnology, Govt. of India (Grant No. BT/IS/002/95-1998–2002).

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REFERENCES


**Notice.** In recognition of his life long contribution for the cause of leprosy, Dr. R. Ganapati, Director, Bombay Leprosy Project (BLP) and the Past President of the Indian Association of Leprologists (IAL) was honored at the 24th Biennial Conference of the IAL in Haldia, West Bengal on 28th February 2004. He received a memento from the Member of Parliament, Mr. Laksman Seth. Dr. Ganapati had also earlier held positions of the Honorary Secretary and Vice President of IAL.

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**Notice from the ILEP.** Three guides which have recently been published by ILEP on the topics of training in leprosy, integration of leprosy services, and how to carry out a skin smear. A brief background to each is given below. These publications can be ordered through books@ilep.org.uk.

1. **ILEP Technical Guide: Training in Leprosy**

   This guide is aimed at staff who organize, support and run leprosy training activities at national, regional or district level. It offers practical guidance on topics such as assessing training needs, effective teaching and learning methods, online learning, on-the-job training and organizing evaluations.

   It has been developed in consultation with a number of practitioners who have extensive experience in leprosy training, and this is reflected in the many practical tools and ideas that it contains. It will be a useful guide for training managers, facilitators, trainers, supervisors and other teaching staff.

   64 pages, 24 cm × 16 cm
   ISBN 0947543260

2. **ILEP Technical Guide: Facilitating the Integration Process—A guide to the integration of leprosy services within the general health system**

   This book offers guidance to public health managers and decision-makers at national and regional level faced with the task of integrating leprosy services into the general health system. The guide systematically describes all the steps involved in the integration process, from situation analysis and the development of a plan of action, to implementation and evaluation.

   It is founded on the experience of countries that have already gone through the integration process, and aims to help ensure that the lessons learned during these experiences are applied more widely.

   36 pages, 24 cm × 16 cm
   ISBN 0947543279

3. **How to do a skin smear examination for leprosy: ILEP Learning Guide**

   This guide consists of three laminated and detachable A4 sheets, and is a clearly presented and durable reference guide for use in the clinic or laboratory. It describes how to carry out all the steps involved in taking a skin smear, and is targeted largely at health workers or laboratory staff with responsibility for taking and reading skin smears, as well as laboratory technicians who may be required to prepare the reagents.

   6 A4 pages in 3 detachable laminated sheets.

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**From IDEA. Leprosy and Human Rights.**

The official discussion of leprosy as a human rights issue continued from May 29 through May 31, 2004 during the 60th Session of the UN Human Rights Commission.
in Geneva, Switzerland. Representatives of The Nippon Foundation, IDEA and the Sasakawa Memorial Health Foundation met with Acting UN High Commissioner Bertrand Ramcharan and also made presentations on the denial of human rights experienced by millions of individuals affected by leprosy and their families. Rights that have been denied include but are not limited to: the Right to Education, the Right to Work, the Right to Freedom of Movement, the Right to Family; the Right to Freedom from Degrading Treatment and the Right to an Existence Worthy of Human Dignity.

For over 3000 years and continuing into the 21st century, the stigma associated with leprosy remains the most persistent and pervasive form of social injustice, prejudice, and discrimination that society has forced upon its fellow human beings. Individuals whose lives have been challenged by leprosy have had their most basic human rights denied by virtually every culture and every major religion throughout time. Throughout this history individuals with leprosy have often been unjustly “blamed” for their disease, with leprosy regarded as punishment for supposed wrongdoing. Discussing leprosy as a human rights issue shifts the burden or responsibility for wrongdoing to society and thus provides a powerful tool for eliminating the stigma and its associated prejudice and discrimination that have denied millions of individuals their rightful place in the world community.

“Our exclusion has been taken for granted in the cultures, religions and languages of society for generations.”

—Arega Kassa Zelelew, IDEA Ethiopia

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The interviewee speaks about the endemic disease, which at present contaminates 4.4 out of ten thousand inhabitants in Brazil, the country with the second highest number of patients. When Tadiana speaks about the Brazilian participation in the program launched by the World Health Organization, she explains that WHO’s objective is to extinguish Hansen’s disease on the planet until 2005. However, she says that Brazilian main goal is to reduce the occurrence of the disease to less than one case per ten thousand people. In our country, the structure and the organization of this program, which comprehends the education and specialization of professionals in order to guarantee early diagnoses, as well as patients’ follow-up during treatment, is developed by Sistema Unico de Saude (SUS) and has been implemented in all the states of the federation. The chemotherapy treatment lasts about a year, when taken seriously. In many cases, the disease comes back a while later. Tadiana comments on the differences of the disease according to the different regions of the country. It has been extinguished in the South, whereas the rates in the North and Central East regions almost reach endemic peaks. Working close to patients and ex-patients associations, first as a nurse and later in the implementation of policies for Hansen’s disease issues, Tadiana Alves Moreira stresses the importance of early diagnoses, which avoid the physical damages and deformities that take place in the advanced stages of the disease, so reducing the stigma over the diseased.—Author’s Abstract


This project presents the complete set of letters between the family of a Hansen’s disease sufferer in the state of Maranhao, in the Northeast of Brazil, and the doctor and bacteriologist Adolpho Lutz. For more than twenty years Fabricio Caldas de Oliveira and Numa Pires de Oliveira, father and son, exchanged a steady flow of letters with the scientist in pursuit of a cure for the disease.

### General and Historical

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disease that had assailed Numa since childhood. The 24 letters compiled here paint a unique portrait of the medical and social drama confronted by this family, and the results of the use of chaulmoogra oil and other medications in their search for alternative treatments.—Authors’ Abstract


During his years of study in Switzerland and Germany, Adolpho Lutz published his first articles on zoology, clinical practice, and therapeutics. In Limeira, Sao Paulo, he began studies on animal and human diseases caused by germs and parasites. In 1885–1886, Lutz traveled to Hamburg to study the morphology of germs related to skin diseases, in conjunction with Paul Gerson Unna, one of Germany’s foremost dermatologists. He proposed the inclusion of Hansen’s and Koch’s bacilli in a new genus. In 1889, Unna nominated his student as physician-in-chief of the Leper Settlement on Molokai Island, Hawaii. From then on, Lutz sustained the theory that the disease was transmitted by mosquitoes. He conducted research to prove this theory when he was head of the Instituto Bacteriologico de Sao Paulo (1893–1908) and, later, after he moved to the Instituto Oswaldo Cruz (1908–1940). Although this research was not successful, on commissions and at congresses in which he participated until his death in October 1940, he still held to his conviction that leprosy was transmitted by mosquitoes.—Authors’ Abstract


From biblical times to the modern period, leprosy has been a disease associated with stigma. This mark of disgrace, physically present in the sufferers’ sores and disfigured limbs, and embodied in the identity of a “leper,” has cast leprosy into the shadows of society. This paper draws on primary sources, written in Spanish, to reconstruct the social history of leprosy in Puerto Rico when the United States annexed this island in 1898. The public health policies that developed over the period of 1898 to the 1930s were unique to Puerto Rico because of the interplay between political events, scientific developments and popular concerns. Puerto Rico was influenced by the United States’ priorities for public health, and the leprosy control policies that developed were superimposed on vestiges of the colonial Spanish public health system. During the United States’ initial occupation, extreme segregation sacrificed the individual rights and liberties of these patients for the benefit of society. The lives of these leprosy sufferers were irrevocably changed as a result.—Author’s Abstract


Deriving funding from missionary sources in Ireland, Britain and the USA, and from
international leprosy relief organizations such as the British Empire Leprosy Relief Association (BELRA) and drawing on developing capacities in international public health under the auspices of WHO and UNICEF through the 1950s, the Roman Catholic Mission Ogoja Leprosy Scheme applied international expertise at a local level with ever-increasing success and coverage. This paper supplements the presentation of a successful leprosy control program in missionary narratives with an appreciation of how international medical politics shaped the parameters of success and the development of therapeutic understanding in the late colonial period in Nigeria.—Author’s Abstract


This article aims to retrieve the history of Hansen’s disease in Brazil, analyzing the medical thinking of the time and the shaping of health policies that permitted the implementation, in Sao Paulo, of a prophylactic policy of compulsory exclusion for all Hansen’s disease patients. It also analyzes how the structuring and implementation of this policy led to a “Sao paulo model” that strongly influenced the rest of the country. It addresses the creation of the state’s network of leper colonies, their characteristics and the emergence of a veritable “parallel state” that endured until 1967, with complete disregard of all the changes taking place in both national and international prophylactic policymaking.—Author’s Abstract


There was a village which was called Yunosawa, lots of leprosy patients lived, existed from 1887 to 1941, Kusatu town, Gunma Prefecture, Japan. It was the only place continued securing self-government to the last as area was free from the isolation policy of State in prewar days there. The aim of this study will make clear the dynamism of “The protection from the tension of the society of leprosy patient currently persecuted” to “The defense of the society from the leprosy patient who is a source of infection.” In this study, explained the factor of confusion to a National Leprosarium Kuryu Rakusen-en during World War II and considered relation between patient movement and residents of Yunosawa village at the postwar period.—Authors’ Abstract


Since the 1920’s, the medical community realized that the strategy of leprosy control based on segregation and persecution of patients was inefficient and expensive. In the 1930’s the new liberal government incorporated leprosy within the general sanitary institutions, by merging the Bureau of Lazarettos and the National Department of Hygiene. The disease-apart approach started to be replaced by a more general public health strategy, which involved controlling other illnesses. Prevention and research played a more influential role, and the new sanitary officials saw leprosy in the light of the economic rationality of expenditures, placing more emphasis on therapies and making them mandatory for all patients. Improvements in leprosy treatment became widely known and available. However, the image of leprosy as a special condition and the practice of segregation were deeply entrenched within the Colombian culture and institutions. The rhetoric changed, but to break with several decades of persecution was a difficult task.—Author’s Abstract

Based on the theories of social representation (SC) and Central Core (CC), a structural study was undertaken regarding the neologism hanseniasis (Hansen’s disease), the term adopted by Brazil’s Ministry of Health in the 1970s. Carried out during 2001, this study interviewed eight hundred housewives residing in the Rio de Janeiro and Duque de Caxias municipalities. It found that Hansen’s disease is part of a process of modernization of common thinking, anchored in the additional representation of leprosy. This finding is understandable from the perspective that the central structure of a social representation has a historical determination, so short- and middle-term changes are not to be expected. Furthermore, there has been no ongoing investment in social marketing to make the new terminology more widely known. The authors discuss the relation between social representation and the concept of the history of mentalities.—Authors’ Abstract


The present paper examines the first attempts to internationalize the problem of leprosy, a subject hitherto overlooked by historians of imperialism and disease. The last decade of the nineteenth century saw many in the “civilized countries” of the imperialist West gripped by a paranoia about an invasion of leprosy via germ-laden immigrants and returning expatriates who had acquired the infection in leprosy-endemic colonial possessions. Such alarmists clamoured for the adoption of vigorous leper segregation policies in such colonies. But the contagiousness of leprosy did not go unquestioned by other westerners. The convocation in Berlin of the first international meeting on leprosy revealed the interplay of differing and sometimes incompatible views about the containment of leprosy by segregation. The roles of officials from several countries, as well as the roles of five protagonists (Albert Ashmead, Jules Goldschmidt, Edvard Ehlers, Armauer Hansen, and Phineas Abraham) in the shaping of the Berlin Conference are here examined.—Author’s Abstract

This article elaborates a significant archival acquisition that supplement the collection documents related to the life and work of Stanley George Browne held at the Wellcome Library for the History and Understanding of Medicine in London, specifically his work in the Belgian Congo (from 1936 to 1959), at Uzuakoli in Nigeria (1959 to 1966), in London with the Leprosy Study Centre (1966–1980), and also in his international capacity as leprosy consultant. It also briefly refers to an endangered collection of documents, photographs, files and correspondence held in a small museum in Culion Sanatorium, The Philippines. This research is part of the International Leprosy Association Global Project on the History of Leprosy. Its results can be accessed at the site http://www.leprosyhistory.org.—Author’s Abstract


In the 1800’s, humoral understandings of leprosy successively give way to disease models based on morbid anatomy, physiopathology, and bacteriology. Linkages between these disease models were reinforced by the ubiquitous seed/soil metaphor deployed both before and after the identification of M. leprae. While this metaphor provided a continuous link between medical descriptions, Henry Vandyke Carter’s On Leprosy (1874) marks a convergence of different models of disease. Simultaneously, this metaphor can be traced in popular medical debates in the late nineteenth century, accompanying fears of a resurgence of leprosy in Europe. Later the mapping of the genome ushers in a new model of disease but, ironically, while leprosy research draws its logic from a view of the world in which a seed and soil metaphor expresses many different aspects of the activity of the disease, the bacillus itself continues to be unreceptive to cultivation.—Author’s Abstract


This article corresponds to part of the results of a research on leprosy-related sources developed in several institutions in the city of Rio de Janeiro. At Real Gabinete Portugues de Leitura, Arquivo Nacional and Biblioteca Nacional, banks, indexes, official documents and photos on the administration of leprosaria and articles on the treatment of the disease have been investigated. At Centro de Pesquisa e Documentacao de Historia Contemporanea do Brazil (CPDOC-FGV), several files have been researched, mainly those with information on the health policies of the first Vargas administration (1930–1945). This research is part of the International Leprosy Association Global Project on the History of Leprosy. Its results can be accessed at the site http://www.leprosyhistory.org—Author’s Abstract


This report is a preliminary result of a survey on memories and history of Hansen’s disease, or ‘hanseniasis,’ prepared by the Fundacao Oswaldo Cruz (Fiocruz) and the Universidade Federal do Rio de Janeiro (UFRJ) using statements from those who have been afflicted by the disease or those that have fought against it. It outlines the methodology used by the authors and gives a succinct history of Hansen’s disease in Brazil, together wish information on the stage of the survey with extracts from our archives of statements. The founding and the role of Movement for the Reintegration of People Afflicted by Hansen’s Disease (Morhan) are explained in the testimony of Thomas Frist, a social scientist who worked in Brazil in the 1970s and 1980s, when the country’s old colonies were being restructured, and Cristiano Torres, a former patient who spent time in prevention centers and leproseries in Para state and who is now active in proposing new policy for the control of Hansen’s disease.—Authors’ Abstract

Soon after the Portuguese made landfall in 1500, Europeans and, later, African slaves introduced leprosy, and Saint Lazarus, the patron saint of its victims, into Brazil. Social and political pressure mounted by the middle of the eighteenth century in the city of Rio de Janeiro to remove those unfortunates from the city’s streets even before the move of Brazil’s capital in 1763. Frei Antoniom the bishop of Rio, founded the venerable hospital that year in the neighborhood of Sao Cristovao. He requested that the Irmandade do Santissimo Sacramento da Candelaria provide oversight and administration. The brotherhood continues to honor its covenant of 239 years ago. The history of this hospital provides insight into the complex relationships that existed between the citizenry and church and state. Rio’s leprosy hospital, now the Hospital Frei Antonio, had an important role in the evolution of the health care professions, progress in medical science, and the genesis of the hygienic movement in Brazil. This study also contributes to the history of a disease that persists in 2002 Brazil as a public health issue.—Author’s Abstract


The national and international agencies working to eliminate leprosy are also dominant in setting the boundaries of official discourse on the issue. Within these boundaries the disease is commonly represented as a medical problem with negative social consequences, and it is believed that both problem and consequences will be resolved if leprosy is eliminated and its victims treated and (if necessary) reintegrated within their social groups. For those affected by leprosy the issues are frequently different, elimination in some respects representing a problem as much as a solution. Against this background, which I describe with reference to a group of leprosy-affected people in South India and their position vis-à-vis leprosy organizations, I explore some of the contexts in which leprosy patients actively manage their own situations, often in defiance of prevailing development orthodoxies. I conclude that closer observation and analysis of the strategies patients use to manage their disease status have important policy implications.—Author’s Abstract


Using molecular methods the authors have studied mycobacterial DNA taken from a 19th century victim of tuberculosis. This was the case from which Robert Koch first isolated and cultured the organism responsible for tuberculosis. The mycobacteria were preserved within five glass culture tubes as abundant bacterial colonies on slopes of a gelatinous culture medium of unknown composition. Originally presented by Koch to surgical laryngologist Walter Johnson Horne in London in 1901, the relic has, since 1983, been in the care of the Royal College of Surgeons of England. Light and electron microscopy established the presence of acid-fast mycobacteria but showed that morphological preservation was generally poor. Eleven different genomic loci were successfully amplified by PCR. This series of experiments confirmed that the organisms were indeed Mycobacterium tuberculosis and further showed that the original strain was in evolutionary terms similar to ‘modern’ isolates, having undergone the TB D1 deletion. Attempts to determine the genotypic group of the isolate were only partially successful, due in part to the degraded nature of the DNA and possibly also to a truncation in the katG gene, which formed part of the classification scheme. Spoligotyping resulted in amplification of DR spacers consistent with M. tuberculosis but with discrepancies between independent extracts, stressing the limitations of this typing method when applied to poorly preserved material.—Authors’ Abstract

Leprosy has definitely been present in China for at least 2000 years. Through painstaking efforts over the past half century, China has put leprosy under control and reached the WHO’s target at elimination of leprosy at the national and subnational level. But difficulties as well as problems like disabilities, discrimination, drug-resistance and dismissing of research still remain in the control of leprosy. Highly attention should be continuously paid on to attain the prevalence rate of less than 0.1/10,000 and the incidence rate below 0.5/100,000 in all counties (cities) throughout the country by the year 2010.—Author’s Abstract

Chemotherapy


We reported a case with acute respiratory distress syndrome (ARDS) caused by rifampicin during therapy for pulmonary tuberculosis. A high level of eosinophil cationic protein in bronchoalveolar lavage fluid (BALF) was detected as well as interleukin-8 and neutrophil elastase. Based on these results together with the positive result of the drug lymphocyte-stimulating test, we concluded that rifampicin was the causative drug leading to ARDS. Corticosteroid therapy resulted in clinical improvement and resolution of the pulmonary infiltrates on the chest radiograph without the recurrence of pulmonary tuberculosis.—Authors’ Abstract


A series of 15 heteroarotinoids has been prepared and evaluated for activity against Mycobacterium bovis BCG with the thiourea-containing isoxyll (7) (0.5 microg/mL) as the standard. 2,2,4-Trimethyl-2H-chromen-7-yl 4-(methoxycarbonyl)benzoate (8) displayed the most significant activity (2.0–4.0 microg/mL) in terms of the lowest concentration (microg/mL) (MIC, minimum inhibitory concentration) required to produce a 99% reduction in the number of colonies on a plate as compared to that system free of the agent at the same dilution of the culture suspension. Ethyl 4-[[N-(2,2,4,4-tetramethylchroman-6-yl)thiocarbamoyl]amino] benzoate (9) and [(1E,3Z,5E)-1-aza-4-methyl-6-(1,2,2,4-tetramethyl(1,2-dihydroquinolyl))hexa-1,3,5-triyl]amino]aminomethane-1-thione (10) exhibited activity at 5.0–10.0 and 10.0–20.0 microg/mL, respectively, while the other examples had MIC values of 20 microg/mL or greater. The inhibitory ability of 8 may occur via the inhibition of mycolic acid synthesis in a like manner as found with 7, but this requires further study. The heteroarotinoids are the first examples to exhibit inhibitory ability against the growth of Mycobacterium bovis BCB.—Authors’ Abstract


OBJECTIVE: To study the changes in methemoglobinemia of 17 children admitted with acute exposure to dapsone complicated by a methemoglobin concentration greater than 20% of the total hemoglobin. The children were treated with multiple doses of activated charcoal with or without the administration of methylene blue. PATIENTS AND METHODS: Seventeen patients (ages 1–13 yrs, median 3 yrs), were admitted 1–72 hr after the ingestion of 100–1200 mg (median 350 mg, 10 patients) or an unknown amount of dapsone
The methemoglobin blood concentrations upon admission ranged from 23.5%–49.7% (median 37.8%), and the main clinical features were cyanosis (17), tachycardia (17), vomiting (11) and tachypnea (8). All of the children received multiple doses of activated charcoal orally or via nasogastric tube (1g/kg, 10% solution, 4–6 times/day, 3–16 doses with a median of 8 doses). Twelve of the 14 patients with methemoglobin levels greater than 30% were also treated with a single dose of methylene blue (1–2% solution, 1–2 mg/kg) infused IV over 5 min. RESULTS: There was a progressive decrease in the methemoglobin levels after the beginning of both treatments (multiple doses of activated charcoal alone or associated with methylene blue), and only one dose of methylene blue was necessary. There were no significant statistical differences between the results of the two treatments according to the time-course decrease in methemoglobinemia (p = 0.49 Wilcoxon test). CONCLUSIONS: Multiple doses of activated charcoal given when methemoglobin levels were greater than 20% can be considered as a possible treatment for pediatric patients, with or without the administration of methylene blue, after acute dapsone exposure.—Authors’ Abstract


Erythema nodosum leprosum (ENL) is a well-known immunological serious complication affecting lepromatous multibacillary leprosy patients. For a long time, ENL has been regarded as an immune complex-mediated disease or Arthus phenomenon. Recently, it has been reported that ENL was associated with high serum tumor necrosis factor-alpha (TNFα) levels, suggesting that this cytokine could also play a central role in the manifestations of ENL. Thalidomide (TH) and systemic steroids (S), both TNFα production inhibitors, are the two current effective drugs for the management of ENL. However, TH is rarely available in leprosy endemic countries, and its teratogenicity and neurotoxicity strongly limit its use. Moreover, the morbidity of S and the frequent steroid-dependence of ENL also create real therapeutic problems. Recently, the efficacy of pentoxifylline (PTX), which also inhibits in vitro and in vivo production of TNFα, has been suggested for ENL treatment. We report our experience on its use for the treatment of 15 leprosy patients suffering from a first ENL attack (11 cases), a chronic steroid-dependent ENL (3 cases) or chronic steroid- and thalidomide-dependent ENL (1 case). PTX has been given at 800 mg t.i.d. (2 cases), or 400 mg t.i.d. (13 cases) doses. The patients received PTX at the initiating dosage until complete clinical cure. At the end of ENL attacks, PTX was either abruptly stopped or tapered down over the next 4 months. In ten of 11 patients who developed ENL for the first time, the systemic symptoms and neuritic pains disappeared within one week; at three weeks, half of the patients were cured and the other half had striking clinical improvement; complete cure was obtained within 7 to 35 days (mean: 27 days). A relapse occurred within 2–3 months in the 5 patients in which PTX was abruptly stopped. In contrast, no relapse occurred in the patients who benefited from decreasing doses of PTX. Recurrent ENL episodes also responded well to PTX. The 3 patients who had chronic steroid-dependent ENL failed to show any improvement after 3 to 6 weeks of PTX. In contrast, steroid therapy could be stopped in the steroid- and thalidomide-dependent patient. Our results confirm the action of PTX if it is slowly tapered down (4 months seem sufficient) and not abruptly to avoid relapses. As it is safe use, PTX could constitute the first line of ENL attack treatment.—Acta Leprologica


There is a renewed interest in thalidomide for use in malignancies and systemic inflammatory diseases. Reduced renal function is not uncommon among patients with these disease states but the pharmacokinetics has not been fully investigated. The aim
of this study was to investigate the pharmacokinetics of thalidomide in haemodialysis patients while on and off dialysis and in myeloma patients with varying degrees of renal function. Two studies were performed. To establish the pharmacokinetics of thalidomide in patients with mild to moderate renal failure, blood samples were taken over 12 weeks from 40 patients with multiple myeloma. A second study was performed in six patients with end-stage renal disease both on a non-dialysis day and before and during a haemodialysis session. Thalidomide concentration was determined by HPLC. A one-compartment open model with first-order absorption and elimination was used to fit total thalidomide concentration to population pharmacokinetics and statistical models using the NONMEM program. Clearance and volumes were slightly below 10 L h−1 and 1 L kg−1, respectively, in both patient groups. The inter- and intrapatient variability was low. Clearance was doubled during dialysis. There was no correlation between thalidomide clearance and renal function. In conclusion, the pharmacokinetics of thalidomide in patients with renal failure are very similar to values reported by others for patients with normal renal function. Although clearance during dialysis is doubled, thalidomide dose need not be changed for patients with decreased kidney function. There is also no need for a supplementary dose due to haemodialysis.—Authors’ Abstract


See Current Literature, Other Mycobacterial Diseases, p. 247.


OBJECTIVES: In order to select new drugs and to predict their in vitro activity against Mycobacterium avium complex (MAC), new quantitative structure-activity relationship (QSAR) models were developed. METHODS: The activities against MAC of 29 structurally heterogeneous drugs were examined by means of linear discriminant analysis (LDA) and multilinear regression analysis (MLRA) by using topological indices (TI) as structural descriptors. In vitro antimycobacterial activities were determined by a broth microdilution method with 7H9 medium. RESULTS: The topological model obtained successfully classifies over 80% of compounds as active or inactive; consequently, it was applied in the search for new molecules active against MAC. From among the selected candidates demonstrating in vitro activity, aflatoxin B1, benzalkonium chloride and pentamidine stand out, with MIC50s between 4 and 32 mg/L. CONCLUSION: The method described in this work is able to select molecules active against MAC.—Authors’ Abstract


Several hypotheses have been proposed to explain the mechanisms of thalidomide teratogenesis, although none adequately accounts for the observed malformations and explains the basis for species specificity. Recent observations that thalidomide increases the production of free radicals and elicits oxidative stress, coupled with new insights into the redox regulation of nuclear transcription factors, lead to the suggestion that thalidomide may act through redox misregulation of the limb outgrowth pathways. Oxidative stress, as marked by glutathione depletion/oxidation and a shift in intracellular redox potential toward the positive, occurs preferentially in limbs of thalidomide-
sensitive rabbits, but not in resistant rats. DNA binding of nuclear factor kappa-B (NF-kappaB), a redox-sensitive transcription factor and key regulator of limb outgrowth, was shown to be significantly attenuated in rabbit limb cells and could be restored following the addition of a free radical spin-trapping agent, phenyl N-tert-butyl nitroine. The inability of NF-kappaB to bind to its DNA promoter results in the failure of limb cells to express fibroblast growth factor (FGF)-10 and twist in the limb progress zone (PZ) mesenchyme, which in turn attenuates expression of FGF-8 in the apical ectodermal ridge (AER). Failure to establish an FGF-10/FGF-8 feedback loop between the PZ and AER results in the truncation of limb outgrowth. We hypothesize that species-selective alterations in redox microenvironment caused by free radical production from thalidomide results in attenuation of the NF-kappaB-mediated gene expression that is responsible for limb outgrowth.—Authors’ Abstract


A 23-year-old woman with linear IgA dermatosis developed dapsone hypersensitivity syndrome (DHS) after initiation of dapsone therapy. She had fever, jaundice with hepatic dysfunction, lymphadenopathy, anemia and dermatitis. The symptoms disappeared with methylprednisolone treatment 40 mg/day.—Journal of Clinical Dermatology


BACKGROUND: Thalidomide is an anti-inflammatory pharmacologic agent that has been utilized as a therapy for a number of dermatologic diseases. Its anti-inflammatory properties have been attributed to its ability to antagonize tumor necrosis factor-alpha (TNF-alpha) production by monocytes. However, its mechanism of action in the skin is not known. PURPOSE: To test our hypothesis that thalidomide may antagonize TNF-alpha production in the skin, we used a mouse model for acute ultraviolet-B (UVB) exposure, a known stimulus for inducing this cytokine. RESULTS: A single bolus dose of thalidomide (either 100 or 400 mg/kg) given immediately before UVB exposure (40–120 mJ/cm²) inhibited, in a dose-dependent manner, sunburn cell formation (i.e., keratinocyte (KC) apoptosis as defined by histologic appearance and confirmed by terminal transferase mediated biotinylated dUTP nick end labelling staining) in mouse skin biopsy specimens. However, this agent did not affect the formation of cyclobutane pyrimidine dimers, a measure of UVB-induced DNA damage, which is an early event associated with apoptosis. RNase protection assays confirmed that high (400 mg/kg), but not low (100 mg/kg), doses of thalidomide inhibited the UVB-induced increase in steady-state TNF-alpha mRNA. Additionally, our in vitro data using neonatal mouse KCs showed that thalidomide prevented UVB-induced cell death (JAM assay). The anti-apoptotic effects of thalidomide can be reversed by the addition of exogenous recombinant mouse TNF-alpha and hence reconstituting UVB-induced programmed cell death. The inhibition of sunburn cell formation by low-dose thalidomide in the absence of TNF-alpha inhibition suggests that other, unidentified mechanisms of apoptosis inhibition are active. CONCLUSIONS: These data suggest that the anti-inflammatory effects of thalidomide can affect UVB injury, and may, in part, explain its action in photosensitivity diseases such as cutaneous lupus erythematosus.—Authors’ Abstract


OBJECTIVES: To examine the effect of first-line and second-line anti-tuberculosis agents on the ability of fluoroquinolones to
kill mycobacteria. METHODS: A clinical isolate of *Mycobacterium tuberculosis* and a laboratory strain of *Mycobacterium smegmatis* were grown in liquid medium and treated with a fluoroquinolone in the presence or absence of anti-tuberculosis agents. Bacterial survival was determined by viable colony counts on agar medium. RESULTS: When moxifloxacin activity was examined in two-drug combinations containing traditional anti-tuberculosis agents, activity was greater than either compound alone with isoniazid, capreomycin and low, but not high, concentrations of rifampicin. Cycloserine contributed no additional activity, and ethambutol interfered with the lethal action of moxifloxacin and gatifloxacin. Experiments with *M. smegmatis* confirmed that both rifampicin and ethambutol reduce fluoroquinolone lethality. Moreover, ethambutol increased the recovery of fluoroquinolone-resistant mutants newly created by ethyl methanesulphonate treatment. CONCLUSIONS: The intrinsic bactericidal activity of C-8-methoxy fluoroquinolones can be adversely affected by some agents currently used for treatment of tuberculosis.—Authors’ Abstract


Patient non-compliance is the major drawback associated with the long-duration chemotherapy of tuberculosis (TB); hence, reduction in dosing frequency forms an important therapeutic strategy. The present study reports the formulation of three frontline antitubercular drugs (ATD), i.e., rifampicin (RIF), isoniazid (INH) and pyrazinamide (PZA) encapsulated in poly (DL-lactide-co-glycolide) (PLG) nanoparticles. Drug encapsulation efficiencies were 56.9 ± 2.7% for RIF, 66.3 ± 5.8% for INH and 68 ± 5.6% for PZA. Following a single oral administration of drug-loaded nanoparticles to *Mycobacterium tuberculosis*-infected mice at every 10th day, no tubercle bacilli could be detected in the tissues after 5 oral doses of treatment. Therefore, nanoparticle-based ATD therapy forms a sound basis for reduction in dosing frequency for better management of TB.—Authors’ Abstract


Thalidomide is being increasingly used in the clinical management of a wide spectrum of immunologically-mediated and infectious diseases, and cancers. However, the mechanisms underlying its pharmacological action are still under investigation. In this regard, oral thalidomide is clinically valuable in the treatment of erythema nodosum leprosum (ENL) and multiple myeloma and effectively reduces tumor necrosis factor-alpha (TNF-alpha) levels and angiogenesis *in vivo*. This contrasts with its relatively weak effects on TNF-alpha and angiogenesis *in vitro* studies and implies that active metabolites contribute to its *in vivo* pharmacologic action and that specific analogues would be endowed with potent activity. Our focus in the structural modification of thalidomide is toward the discovery of novel isosteric active analogues. In this regard, a series of thiothalidomides and analogues were synthesized and evaluated for their TNF-alpha inhibitory activity against lipopolysacharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC). This was combined with a PBMC viability assay to differentiate reductions in TNF-alpha secretion from cellular toxicity. Two isosteric
analogues of thalidomide, compounds 15 and 16, that mostly reflect the parent compound, together with the simple structure, dithioglutaramide 19, potently inhibited TNF-alpha secretion, compared to thalidomide, 1. The mechanism underpinning this most likely is posttranscriptional, as each of these compounds decreased TNF-alpha mRNA stability via its 3′-UTR. The potency of 19 warrants further study and suggests that replacement of the amide carbonyl with a thiocarbonyl may be beneficial for increased TNF-alpha inhibitory action. In addition, an intact phthalimido moiety appeared to be requisite for TNF-alpha inhibitory activity.—Authors’ Abstract

Clinical Sciences


Histoid leprosy is a particular variant of lepromatous leprosy presenting as cutaneous or subcutaneous nodular and/or plaque-like lesions arising from apparently normal skin. It is characterized histologically by spindle-shaped histiocytes in interlacing bundles and whorls, containing numerous intact and rod-shaped *Mycobacterium leprae*. It can occur *de novo* or secondary in patients treated for a long course by dapsone alone. We describe a case of lepromatous leprosy treated according to the national Moroccan protocol who developed histoid lesions during his treatment by dapsone. The patient responded well to fluoroquinolone, rifampicin and clofazimine, with however, the occurrence of erythema nodosum leprosum.


Leprosy (Hansen’s disease) causes the most common treatable form of neuropathy in the world. Several endemic countries account for the majority of the world’s cases and most of the cases seen in the US are amongst immigrants. However, endemic cases of leprosy occur in the US. The pathogen is *Mycobacterium leprae*, a slow-growing, obligate intracellular pathogen that consistently infects skin and peripheral nerves. The clinical appearance of the skin and neurologic deficits develop months to years after infection and are determined by the host’s response to the infection. An individual’s disease classification can change over time based on the immune status of the individual. Immune-mediated “reactional states” may also occur that require additional recognition and treatment. Varied in its manifestations, a successful treatment approach relies on proper recognition and classification of disease.—Author’s Abstract


The number of registered leprosy patients world-wide has decreased dramatically after extensive application of WHO recommended Multiple Drug Therapy (MDT). The annual number of new cases has, however, been almost unchanged in several populations, indicating that the infection is still present at community level. Nasal carriage of *Mycobacterium leprae* DNA was studied in Lega Robi village in Ethiopia. MDT had been applied for more than ten years, and 718 residents over 5 years old were eligible for the study. During the first survey nasal swab samples were collected from 664 (92.5%) individuals. The results of a Peptide Nucleic Acid-ELISA test for *M. leprae* DNA interpreted by stringent statistical criteria were available for 589 (88.7%) subjects. Thirty-five (5.9%) individuals without clinical signs of leprosy were positive for *M. leprae* DNA. Seven PCR positive individuals lived in a household where one or two other members were also positive for *M. leprae* DNA. Seven PCR positive individuals lived in a household where one or two other members were also positive for *M. leprae* DNA. During a second survey 8 (46%) of 175 interpretable PNA-ELISA tests were positive. Of 137 individuals tested twice, only two were positive on both occasions whereas 10 were
PCR positive only once. The study confirms the widespread distribution of *M. leprae* DNA in healthy individuals. The feasibility of curbing possible transmission of subclinical infection needs further consideration.


Leprosy among children is a public health problem reflecting the disease’s transmission in the community and the efficiency of control programmes. 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 percent). Forty-seven percent of children were 10 years old or more; 28.5 percent shared their dwellings with leprosy patients; 35 percent had only one lesion, and 43 percent were multibacillary cases. Agreement between clinical and histopathological classification was 36 percent; hypochromic chronic eczema and post-inflammatory incontinence of melanin pigment were the clinical lesions most frequently mistaken with leprosy. Leprosy among children represents 7 percent 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.—Authors’ Abstract


We evaluated a patient with disseminated *Mycobacterium tuberculosis* and *Mycobacterium chelonae* infection, of which he died. He also developed autoimmune (type I) diabetes and primary hypothyroidism. His serum contained a high titer of immunoglobulin G autoantibody to interferon-gamma (IFN-gamma) capable of blocking *in vitro* responses to this cytokine by peripheral blood mononuclear cells from normal donors. These results suggest that autoantibodies to IFN-gamma can induce susceptibility to disseminated mycobacterial infection, which may be refractory to chemotherapy.—Authors’ Abstract


**INTRODUCTION:** Despite prevention programs, tuberculosis is still progressing endemically in developing countries. The prevalence of cutaneous tuberculosis is estimated as 2.1 p. 100 and represents a rare localization among the extra-pulmonary forms. In order to study the epidemiology, the most frequent anatomoclinical forms and the progressive features of cutaneous tuberculosis, we conducted a study in the area of Tunis over a 20-year period. PATIENTS AND METHODS: All cases of cutaneous tuberculosis observed between 1981 and 2000 in the dermatology department of the Habib Thameur hospital were included in a retrospective study. Diagnosis of cutaneous tuberculosis was challenging and required the correlation of clinical, biological and progressive features. RESULTS: Twenty-six patients were observed in the study. There were 12 men and 14 women with a mean age of 30.4 years (range: 6 to 74) and 20 p. 100 of infantile cases. Of the various patterns of cutaneous tuberculosis seen, 11 (42 p. 100) had lupus tuberculosis, 10 (38 p. 100) had scrofuloderma, 4 (15 p. 100) had tuberculosis verrucosa cutis and 1 child had a perianal tubercular ulcer. The Mantoux test was positive in 20/24 patients. Histological tuberculoid granuloma was seen in 25 cases
(96 p. 100) associated with caseating necrosis in 10 cases (38 p. 100). All patients were treated successfully with triple or quadruple anti-tubercular drugs for 6 to 10 months. One patient exhibited a squamous cell carcinoma on a lupus tuberculosis scar four years later. DISCUSSION: The progression of cutaneous tuberculosis remains stable, ranging from 1.4 cases/year between 1981 and 1990 to 1.2 cases/year between 1991 and 2000. In our study, females were slightly more affected than men with a M/F sex ratio of 0.86. Before 1984, scrofuloderma was the most frequent form among the cutaneous tuberculoses. Now the frequency of lupus tuberculosis has reached that of scrofuloderma, demonstrating the increase in the incidence of clinical pattern of cutaneous tuberculosis with strong immunity probably related to the improvement in health conditions and generalization of vaccination programs.—Authors’ Abstract


During the last 20 years, the global leprosy situation has strikingly changed with a decrease of cases from 12 millions estimated cases in 1982 to 600,000 registered cases in the year 2000. However, during the past 15 years, about 700,000 new cases are still detected annually. The systematic use of multidrug therapy (MDT), as recommended by a WHO Study Group in 1982, has proven its efficacy as assessed by the low reported relapse rate (less than 1% per year). The initial PCT schedule has been modified several times, but this PCT remains the recommended chemotherapy for the great majority of patients. New potent antibacillary drugs (ofloxacin, minocycline, clarithromycin) have been discovered; however, their current use is limited and should remain limited until under way trials could confirm their efficacy. With the use of PCT, the frequency of immunologically mediated reaction states have changed. The occurrence of reversal reaction (type 1 reaction), has significantly increased while that of erythema nodosum leprosum (ENL, type 2) appeared less common. Because of the high risk of neurological permanent damage, reversal reaction needs to be diagnosed and treated as soon as possible. Herein, the current antibacillary and antireactional treatments are being reviewed.—Bulletin de la Société de Pathologie Exotique


The clinical diagnosis of pure neural leprosy (PNL) remains a public health care problem mainly because skin lesions—the cardinal features of leprosy—are always absent. Moreover, the identification of the leprosy bacillus is not easily achieved even when a nerve biopsy can be performed. In an attempt to reach a reliable PNL diagnosis in patients referred to our Leprosy Outpatient Clinic, this study employed a variety of criteria. The nerve biopsies performed on the 67 individuals whose clinical, neurological, and electrophysiological examination findings strongly suggested peripheral neuropathy were submitted to M. leprae identification via a polymerase chain reaction (PCR). Mononeuropathy multiplex was the most frequent clinical and electrophysiological pattern of nerve dysfunction, while sensory impairment occurred in 89% of all cases and motor dysfunction in 81%. Axonal neuropathy was the most prominent electrophysiological finding, while the histopathological nerve study showed epithelioid granuloma in 14% of the patients, acid fast bacilli in 16%, and nonspecific inflammatory infiltrate and/or fibrosis in 39%. PCR for M. leprae was positive in 47% of the nerve biopsy samples (n =23). PCR, in conjunction with clinical and neurological examination results, can be a powerful tool in attempting to identify and confirm a PNL diagnosis.—Tropical Disease Bulletin

Matsuo, E. [The sequelae of Hansen’s disease. (Pathologic viewpoint of etiologies,
The proportion of glomerulonephritis, often a sequence of arteriolitis, among the sequelae of Hansen’s disease after the introduction of chemotherapy increased markedly in Japan and nullified that of once prevalent tuberculosis after 1960s. However, most significant aftermath of the disease for numbers of years in the past have been peripheral nerve injuries worldwide for which effective countermeasures are yet to be developed. In this brief autopsy cases study from 1960s to 1990s, we confirmed the presence of cases in which arteriolitis and resulted infarction of peripheral nerves and not \textit{M. leprae} itself were shown to be the major cause of axonal damages. There were also cases in which the accumulation of the bacilli without vascular changes did not damage the axons. The cases as these could not be solitary but should be rather common in this time of chemotherapy. If so, the methods to reconstruct nerves and blood vessels by promoting those regeneration should be developed to cope with the situation for surgeon, assisted by pathologists.—Authors’ Abstract


Erythema nodosum (EN) is seen only in the primary tuberculosis (TB) form of tuberculous diseases. Among the etiologies of EN, TB is the most frequent disorder in developing countries. We aimed to assess our patients with EN in reference to primary TB. We evaluated 335 patients with the diagnosis of TB during last 20 years; retrospectively 61 (18\%) of these cases had pulmonary and 274 (82\%) had extrapulmonary TB. Ten (16\%) of the pulmonary TB cases were primary. All 10 patients with primary TB presented with EN. Among 50 patients with EN diagnosed and followed during the last 10 years, the etiology was determined in 56\%, and primary TB was the most frequent: 20\%.—Authors’ Abstract


Much evidence exists on pulmonary tuberculosis (PTB) as a presenting feature of HIV infection or AIDS-related complex, while few reports exist of a direct association between HIV infection and leprosy.
This study was carried out to see whether or not an association between leprosy and HIV infection existed, similar to that of PTB in the region of Maiduguri, Nigeria. Of 105 patients with leprosy, 11 (10.5%) were positive for HIV antibody. Of 58 patients with suspected PTB, 11 (19%) were positive for HIV antibody. Twenty-seven (47%) of the 58 had active PTB, with results of sputum smear and culture positive for mycobacterium, and six of these (22.2%) were also positive for HIV antibody. Odds ratios (OR) obtained by conditional logistic regression (matched) analysis were 3.52 (95%, CI 1.03–12.07) and 2.53 (95%, CI 1.04–6.15) for association between HIV-1 and PTB and leprosy, respectively. HIV infection was more prevalent among leprosy patients aged under 30 years, OR = 4.25 (95%, CI 1.25–14.42). The prevalence of HIV-1 infection was at borderline significance, higher in PTB and leprosy patients than in blood donors, Fisher’s exact test (two-tailed) p = 0.07 and p = 0.05, respectively.


BACKGROUND: Disabilities constitute the main problem of leprosy. It is important to identify risk factors involved, so it can be possible the prone patients be followed-up more carefully.

OBJECTIVES: To determine if the presence of thick and/or painful peripheral nerves at diagnosis correlates with disabilities already present at the initial examination, as well as with subsequent development of neuritis, during and after multidrug therapy.

METHODS: One hundred and three patients with multibacillary forms of leprosy were studied and we noted the presence of compromised peripheral nerves at diagnosis, the disability grade before treatment (DGBT), and the occurrence of neuritis episodes during and after multibacillary multidrug therapy.

RESULTS: The detection of affected peripheral nerves at diagnosis, correlated statistically (p <0.005) with the occurrence of disabilities (DGBT >0). It also correlated significantly with the development of neuritis in the follow-up (average of 64.6 months from diagnosis, during and after multidrug therapy).

CONCLUSIONS: We emphasize the need of a good examination of peripheral nerve trunks in multibacillary patients at the diagnosis, in order to improve the detection of disabilities already present, and specially to prevent further disabilities. Healthy professionals who deal with leprosy patients must be aware to the initial neurological impairments because those patients are more susceptible to the occurrence of neuritis and neurological sequelae.—Anais Brasileiros de Dermatologia


Generalized adenopathy as a manifestation of type 2 reactional leprosy Leprosy patient’s reactions are severe clinical manifestations of acute inflammation of chronic lesions, capable of producing irreversible and invalidating damage. We studied a 46 year-old man with a type 2 leprosy reaction, who presented fever, cutaneous nodules, nasal obstruction and generalized adenopathy. The hemogram showed leucocytosis with neutrophilia. None of the initial diagnoses included leprosy. A lymph node biopsy revealed extensive necrotic areas infiltrated with polymorphonuclear lymphocytes, and foamy macrophages. Eosinophilic necrosis and thrombosis of venules with lymphoid nodule depletion was also in evidence. Ziehl Neelsen stain was not done, but the Gomori stain clearly showed Hansen’s bacilli. These were not detected by the pathologist and therefore a final diagnosis was not provided. Twenty months later, the patient presented similar symptoms, but with more generalized lymphoid nodule depletion was also in evidence. Ziehl Neelsen stain was not done, but the Gomori stain clearly showed Hansen’s bacilli. These were not detected by the pathologist and therefore a final diagnosis was not provided. Twenty months later, the patient presented similar symptoms, but with more generalized lymphoadenopathy and presence of cutaneous nodules. Nodule biopsy showed lepromatous leprosy with erythema nodusum leprosum or type 2 reaction. Polychemotherapy treatment and anti-reaction treatment with
thalidomide cured the patient. No sequelae were noted in 3 years following the treatment. A literature review of the type 2 reaction in leprosy is provided, including discussion of risk factors, histopathology, differential diagnosis for leprosy adenopathy, pathogenesis, prognosis, and treatment. Type 2 leprosy must be treated immediately upon diagnosis as it can cause serious and permanent tissue damage. As had occurred in the above patient, the disease can proceed with generalized and symptomatic lymphadenopathy.—Authors’ Abstract


Mycobacterium leprae (M. leprae), the causative agent of Hansen’s disease, is endemic in many areas of Asia, sub-Saharan Africa, South and Central America, the Pacific Islands, and the Philippines. The spectrum of clinical disease is dependent on the patient’s cell-mediated immunity and might range from localized anesthetic patches or plaques to disseminated disease. If undiagnosed, progression with damage to the involved sensory and motor nerves might occur. Lepromatous vasculitis occurs most commonly in patients with severe disseminated disease. Vascular disease, as the initial presenting sign of tuberculoid leprosy, is, however, rare. We present one patient in whom the development of Hansen’s disease was associated with involvement of the external jugular vein and was initially seen as external jugular vein fibrosis.—Authors’ Abstract


It is generally accepted that tuberculosis results from a single infection with a single Mycobacterium tuberculosis strain. Such infections are thought to confer protective immunity against exogenous reinfection. In this study, a novel polymerase chain reaction method was developed to specifically identify M. tuberculosis strains belonging to the Beijing and non-Beijing evolutionary lineages in sputum specimens collected from tuberculosis patients resident in an epidemiologic field site in Cape Town, South Africa. The sensitivity and specificity of the polymerase chain reaction-based strain classification method were 100% (95% confidence interval, 85–100%) when compared with DNA fingerprinting and spacer oligotyping (spoligotyping). Application of this method showed that 19% of all patients were simultaneously infected with Beijing and non-Beijing strains, and 57% of patients infected with a Beijing strain were also infected with a non-Beijing strain. Multiple infections were more frequent in retreatment cases (23%) as compared with new cases (17%), but were not associated with sex, age, or smear grading. These results suggest that multiple infections are frequent, implying high reinfection rates and the absence of efficient protective immunity conferred by the initial infection. This finding could influence our understanding of the epidemiology of disease in high-incidence regions and our understanding for vaccine development.—Authors’ Abstract


OBJECTIVE: To investigate feasible treatment methods for plantar ulcers in leprosy patients according to the agreement between the Ministry of Health (MOH) of China and the Leprosy Mission International (LMI). METHODS: A total of 2599 complicated foot ulcers in 1804 leprosy cases underwent surgic treatment. Plastic fixation and supports were used, dressings were changed regularly, and protective footwear and modified insoles were provided. RESULTS: Of the 2599 foot ulcers 1446 (55.64%) healed. The cure rate of the patients treated in leprosy hospitals was 71.31%, with 219 (15.15%) recurrences of foot ulcers. The recurrence rate of those who
lived at home was 18.35%. CONCLUSIONS: Comprehensive treatment of foot ulcers has a high cure rate and a low recurrence rate. Reduction of workload, avoidance of long distance walking, intensification of education on foot self-care and provision of financial support are the main measures for preventing a recurrence of foot ulcers.—Authors’ Abstract


Mycobacterial infections of macrophages have been shown to inhibit the ability of the macrophage to respond to IFN-gamma. We previously reported that Mycobacterium avium infection of mouse macrophages decreases IFN-gamma-induced STAT1 tyrosine phosphorylation and STAT1 DNA binding. Because macrophages respond to M. avium through Toll-like receptor 2 (TLR2), we determined whether TLR2 stimulation inhibits the response to IFN-gamma. Treatment of mouse RAW264.7 macrophages with TLR2 agonists inhibited the induction of IFN-gamma-inducible genes by IFN-gamma. In contrast to M. avium infection, TLR2 agonists did not inhibit the IFN-gamma induction of DNA-binding activity of STAT1 and the tyrosine phosphorylation of STAT1alpha. Instead, IFN-gamma induction of RAW264.7 cells treated with TLR2 agonists resulted in an increase in the tyrosine phosphorylation of the dominant-negative STAT1beta. TLR2 stimulation of RAW264.7 cells increased both STAT1beta protein and mRNA expression, suggesting that the increased STAT1beta phosphorylation results from increased STAT1beta expression. Because STAT1alpha and STAT1beta mRNA have different 3’ untranslated regions, and 3’ untranslated regions can regulate mRNA stability, we examined the effects of TLR2 stimulation on mRNA stability. TLR2 stimulation of RAW264.7 cells increased the stability of STAT1beta mRNA, while not affecting the stability of STAT1alpha mRNA. The ability of STAT1beta to function as a dominant negative was confirmed by overexpression of STAT1beta in RAW264.7 macrophages by transient transfection, which inhibited IFN-gamma-induced gene expression. These findings suggest that M. avium infection of mouse macrophages inhibits IFN-gamma signaling through a TLR2-dependent increase in STAT1beta expression by mRNA stabilization and a TLR2-independent inhibition of STAT1 tyrosine phosphorylation.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 255.


Heat shock proteins (HSP) have been shown to enhance antigen processing and presentation through their association with antigenic peptides and delivery of these moieties into major histocompatibility complex class I pathways. In this study, mycobacterial Hsp65 is demonstrated to have the ability to help cross-present an exogenous protein by dendritic cells (DC) to CD8 T cells without the need for complex formation between Hsp65 and the protein. This ability of Hsp65 to enhance cross-presentation is independent of its weak stimulatory effect on DC, the latter seen only after prolonged incubation. When the effect of lipopolysaccharide contamination is abrogated, Hsp65 is unable to activate Toll-like receptor (TLR)4 in the
presence of CD14 and MD2. This accounts for the inability of Hsp65 to drive maturation of DC and shows that Hsp65 is not a potent stimulator of DC. Thus, Hsp65 enhances the cross-presentation of a soluble, free antigen by DC, independent of TLR4 signaling and up-regulation of costimulatory molecules.—Authors’ Abstract


The pathogenesis of tuberculosis (TBC) meningitis is still unknown. As shown by previous studies, human microglia can be the target of mycobacteria, but no data are available about their cellular response to infection. Consequently, we studied the expression of tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) and IL-10 in human microglia pure cultures infected with the two variants of Mycobacterium avium (domed-opaque (SmD) and transparent (SmT)) and with Mycobacterium tuberculosis. Results showed that microglia was productively infected by mycobacteria which could grow inside the cells. Mycobacteria internalization was more rapid for M. avium, but M. tuberculosis infection turned out to be more efficient due to the incorporation of densely packed bacteria. TNF-alpha expression was not affected by M. avium, whereas an increase followed by a decrease was observed in M. tuberculosis. Both IL-1 and IL-10 cytokine expression was rapidly inhibited by infection with the more virulent bacteria, whereas the non-pathogenic one had almost no effect. Also, the expression of the co-stimulatory molecule CD137, a member of tumor necrosis factor receptor family, was affected by infection with virulent mycobacteria. Our results show that microglia response to mycobacterial infection is modulated in correlation with virulence, mainly toward inhibition of inflammatory response. This observation might be one of the mechanisms by which non-pathogenic mycobacteria are quickly eliminated, explaining one of the bases of virulence.—Authors’ Abstract


Thalidomide is an effective treatment for several inflammatory and autoimmune disorders including erythema nodosum leprosum, Behcet’s syndrome, discoid lupus erythematosus, and Crohn’s disease. Thalidomide is believed to exert its anti-inflammatory effects, at least in part, by inhibiting tumor necrosis factor-alpha (TNF-alpha) production by monocytes. We studied the effects of thalidomide on epidermal Langerhans cells (LC). LCs are epidermal antigen-presenting dendritic cells that play important roles in skin immune responses. Using the murine epidermis-derived dendritic cell lines, XS106A from A/J mice and XS52 from BALB/c mice as surrogates for LC, we found that thalidomide inhibited TNF-alpha production in a concentration-dependent manner. Northern blot analysis revealed that thalidomide significantly decreased the peak-induced mRNA level of TNF-alpha in XS106A cells and XS52 cells. We then examined the effect of thalidomide on fresh LC enriched to approximately 98% using positive selection of Ia+ cells with antibodies conjugated to magnetic microspheres. TNF-alpha production was reduced by 67.7% at a thalidomide concentration of 200 microg per mL. Thalidomide also had a profound inhibitory effect on the ability of LC to present antigen to a responsive TH1 clone. Thalidomide inhibits TNF-alpha production and the antigen-presenting ability of epidermal LCs. These mechanisms may contribute to the therapeutic effects observed with this agent.—Authors’ Abstract

As a result of damaging endothelial cells (ECs), *Mycobacterium leprae* triggers the production of antibodies (Abs). These anti-EC Abs (AECAs) can be divided into two types. The first type nonspecifically reacts with components of the cytosol (CY) and can be detected by enzyme-linked immunosorbent assay (ELISA). The second specifically reacts with the EC membrane (MB) and requires fluorescence-activated cell sorter (FACS) analysis to be detected. The presence of both types of AECAs was determined in 68 leprosy patients. The ELISA was positive for 35 of them but also for 30 of 34 malaria patients and 17 of 50 healthy African controls. However, whereas FACS analysis showed MB reactivity in only three malaria patients and four controls, this reactivity was found in 27 leprosy patients, more of those having the lepromatous than the tuberculoid form. Specificity for MB, which we failed to absorb by incubation with CY lysates, predominated over that for CY in leprosy, unlike malaria, where the EC reactivity was restricted to the CY. Western blot analysis and two-dimensional electrophoresis revealed that calreticulin, vimentin, tubulin, and heat shock protein 70 were targeted by AECAs from leprosy patients, but other proteins remained unidentified. These auto-Abs, but not those from malaria patients, did activate ECs, as indicated by the E-selectin and intercellular adhesion molecule 1 upregulation, and/or induced them into apoptosis, as documented by four different methods. Our findings suggest that, in some but not all leprosy patients, AECAs may play a role in pathogenesis.—Authors’ Abstract


*Mycobacterium tuberculosis* culture filtrate protein-10 (CFP-10) (Rv3874) is considered a promising antigen for the immunodiagnosis of tuberculosis (TB) together with early secreted antigens of *M. tuberculosis* (ESAT-6). Both ESAT-6 and CFP-10 are encoded by the RD1 region that is deleted from all tested *M. bovis* bacille Calmette-Guerin (BCG) strains but present in *M. leprae*, *M. tuberculosis*, *M. bovis*, *M. kansasii*, *M. africanum* and *M. marinum*. In this study, the homologue of CFP-10 in *M. leprae* (ML0050) is identified and characterized. Interferon-gamma production in response to this homologue by T cells from leprosy patients, TB patients and unexposed controls shows that CFP-10 of *M. leprae* is a potent antigen that crossreacts with CFP-10 of *M. tuberculosis* at the T-cell level. This crossreactivity has implications for the use of CFP-10 as a diagnostic marker for leprosy and tuberculosis.


Sarcoidosis is a multisystem granulomatous disorder of unknown origin characterized by the presence of epithelioid granulomas in the affected organs. Histological and clinical similarities between sarcoidosis and tuberculosis caused by *M. tuberculosis* suggest a shared underlying pathophysiology. However, specific markers are needed. This study examined the differential gene expression pattern in alveolar macrophages of patients with granulomatous disorders. The differential mRNA regulation pattern of alveolar macrophages in the bronchoalveolar lavage of healthy controls was compared to that of patients with sarcoidosis and tuberculosis by means of differential display reverse transcription PCR. Comparative analysis of 2498 PCR products in controls, sarcoidosis, and tuberculosis revealed a differential regulation of expressed sequence tags in only 6.5%. 1.8% showed a shared expression pattern between sarcoidosis and tuberculosis in contrast to the control. It can be assumed that these alterations are associated with common granulomatous features. In contrast, 3.0% of the amplified sequence tags showed specific up- or downregulation in sarcoidosis and 1.6% in tuberculosis. These data indicate a significant proportion of common granuloma-associated features, independent of the origin of the granulomatous disorder.—Authors’ Abstract

The resurgence in mycobacterial infection worldwide has led to renewed attention to the pathogenesis of Mycobacterium species. Although interferon-gamma (IFN-gamma) is a principal mediator of macrophage activation, macrophages infected with Mycobacterium are poor in response at the cytokine. However, the molecular mechanisms underlying mycobacterial infection remain unclear. The purpose of this study was to elucidate the mechanism of the poor response to IFN-gamma in mycobacterial infection. Our data clearly demonstrate that this is due to induction of suppressor of cytokine signal (SOCS) negative regulators of IFN-gamma signal transduction that closely correlates with the inhibition of JAK/STAT signaling and gene expression stimulated by IFN-gamma. Mycobacterium bovis bacillus Calmette-Guerin infection induces the production of SOCS-1 and SOCS-3 in murine J774 macrophages. The level of SOCS-1 mRNA increased 1 h and reached a maximum 3 h after the addition of the bacteria. SOCS-3 mRNA expression appeared as early as 1 h after the infection. We also observed that trehalose 6,6′-dimycolate/cord factor, a major component of the Mycobacterium tuberculosis cell wall, induces expression of SOCS and inhibits IFN-gamma-stimulated phosphorylation of STAT1 extensively in the cells. The results in this study suggest that a molecular mechanism of mycobacterial infection affects the unresponsiveness to IFN-gamma in the subsequent growth and spread of macrophages.—Authors’ Abstract


Members of the CD1 family present antigenic lipids to T lymphocytes. CD1 molecules survey endocytic compartments for lipid antigens that are sorted into these vesicles after incorporation into the membrane bilayer, and extraction from the bilayer is likely to be a critical step for lipid association. We hypothesized that lysosomal saposins, which are cofactors required for sphingolipid degradation, might be involved in this process. Here we show that saposins, although not required for the autoreactive recognition of CD1d by natural killer T cells, are indispensable for the binding of an exogenous lipid antigen, α-galactosylceramide, to CD1d in the endocytic pathway. We suggest that saposins mobilize monomeric lipids from lysosomal membranes and facilitate their association with CD1d.—Nature Immunology


The role of mitogen-activated protein kinase (MAPK) signaling pathways in the regulation of TNF-alpha and NOS2 production by human monocytes infected with Mycobacterium bovis BCG was examined. Inhibition studies showed that ERK1/2 and p38 MAPK activation were necessary for the monocyte response to M. bovis infection. Analysis of MAPK activation showed rapid phosphorylation of ERK1/2 and p38 in response to M. bovis BCG. Phosphorylation was not due to an autocrine effect of TNF-alpha secretion, since an anti-TNF-alpha antibody had no significant effect on the levels of p38 phosphorylation. The inhibitor PD98059 significantly reduced M. bovis BCG-induced TNF-alpha production and almost completely abrogated phosphorylation of ERK1/2; in addition the potent MEK inhibitor U0126 also abrogated phosphorylation. In contrast, studies using inhibitors selective for ERK1/2 and p38 showed that p38
plays an essential role in the induction of NOS2, whereas the role of ERK1/2 was minor. These results suggest that ERK1/2 and p38 kinases differentially regulate the *M. bovis* BCG-mediated induction of TNF-alpha and NOS2 in human monocytes.—Authors’ Abstract


Gammadelta T lymphocytes are involved in a great variety of inflammatory and infectious responses. However, the mechanisms by which gammadelta T lymphocytes migrate to inflamed sites are poorly understood. In this study we investigate the role of monocyte chemotactic protein (MCP)-1 in regulating gammadelta T cell migration after LPS or *Mycobacterium bovis* bacille Calmette-Guerin (BCG) challenge. LPS-induced gammadelta T cell influx was significantly inhibited by either pretreatment with dexamethasone or vaccinia virus Lister 35-kDa chemokine binding protein, vCKBP, a CC chemokine neutralizing protein, suggesting a role for CC chemokines in this phenomenon. LPS stimulation increased the expression of MCP-1 mRNA and protein at the inflammation site within 6 hr. It is noteworthy that LPS was unable to increase MCP-1 production or gammadelta T cell recruitment in C3H/HeJ, indicative of the involvement of Toll-like receptor 4. Gammadelta T cells express MCP-1 receptor CCR2. Pretreatment with anti-MCP-1 mAb drastically inhibited LPS-induced in vivo gammadelta T cell mobilization. Indeed, MCP-1 knockout mice were unable to recruit gammadelta T cells to the pleural cavity after LPS stimulation, effect that could be restored by coadministration of MCP-1. In addition, BCG-induced gammadelta lymphocyte accumulation was significantly reduced in MCP-1 knockout mice when compared with wild-type mice. In conclusion, our results indicate that LPS-induced gammadelta T lymphocyte migration is dependent on Toll-like receptor 4 and sensitive to both dexamethasone and CC chemokine-binding protein inhibition. Moreover, by using MCP-1 neutralizing Abs and genetically deficient mice we show that LPS- and BCG-induced gammadelta T lymphocyte influx to the pleural cavity of mice is mainly orchestrated by the CC chemokine MCP-1.—Authors’ Abstract


*Mycobacterium tuberculosis* (MTb) is the leading cause of death in the setting of AIDS. MTb enhances the pathogenicity and accelerates the course of HIV disease and, furthermore, infection with HIV-1 increases the risk of reactivation or reinfection with MTb. In this study, we show that host-specific recall responses to one pathogen, MTb, has a direct effect upon the regulation of a second pathogen, HIV-1. Using cells from immunocompetent former tuberculosis (TB) patients who displayed either a persistently positive (responsive) or negative (anergic), delayed-type hypersensitivity (DTH) reaction to intradermal injection of purified protein derivative (PPD), we investigated the effect of recall Ags to MTb upon the replication of HIV-1 primary isolates in vitro. We show that HIV-1 replication of a T cell-tropic isolate was significantly impaired in MTb-stimulated PBMC from PPD-anergic donors. Furthermore, these donors displayed a significant increase in CD8(+) T cells and IL-10 levels and lower levels of IL-2 and TNF-alpha relative to PPD-responsive donors in response to PPD stimulation. Strikingly, CD8(+) T cell depletion and blocking of IL-10 significantly increased HIV-1 replication in these PPD-anergic donors, indicating that an immunosuppressive response to MTb recall Ags inhibits HIV-1 replication in PPD-anergic individuals. Therefore, immunotherapeutic approaches aimed at recapitulating Ag-specific MTb energy in vivo could result in novel and effective approaches to inhibit HIV-1 disease

*Mycobacterium avium* complex (MAC) adheres, invades and multiplies inside epithelial cells. Earlier, we demonstrated two MAC protein adhesins, 25 and 31 kDa, binding with HEp-2 cells. The 25 kDa MAC adhesin was found to be superoxide dismutase (SOD). In this study, epithelial cell (HEp-2 and A549) ligands for MAC-SOD were identified by probing two-dimensional western blots of epithelial extracts with MAC proteins followed by monoclonal anti-MAC-SOD antibodies. Three epithelial cell proteins with molecular masses 43, 40 and 18 kDa, present in both membrane and cytosolic fractions, were found to bind with MAC-SOD. Based on the N-terminal amino acid sequences, the 43, 40 and 18 kDa epithelial proteins were identified as aldolase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and cyclophilin A (CypA), respectively. Furthermore, MAC-SOD was found to bind to purified rabbit muscle aldolase, GAPDH and recombinant CypA in western blotting.—Authors’ Abstract


Standard PCR-based detection of mycobacterial DNA in paraffin-embedded specimens may lack sufficient sensitivity because of the degradation of nucleic acids caused by routinely used formalin fixation. Therefore, we set up an approach that aimed at improving the results by applying the novel HOPE-fixative in PCR-detection of mycobacteria in paraffin-embedded tissues. Comparison of PCR-results using DNA extracted from either HOPE- or formalin-fixed specimens in BCG-infected SCID-mice revealed a more than 100fold enhanced sensitivity for the HOPE-fixed material. Owing to the preservation of DNA from degradation in HOPE-fixed tissues, even differentiation within the *M. tuberculosis* complex was possible by spoligotyping. We therefore conclude that the HOPE-fixative is a useful tool for molecular pathology that enhances
the sensitivity of PCR-based methods for the detection of pathogens in paraffin-embedded tissues compared to formalin-fixation. Owing to the better preserved DNA, improved differentiation of mycobacteria from archived materials is possible. These results promise new and a substantially wider range of possibilities in the field of molecular pathology.—Authors’ Abstract


Continuing our research on Mycobacteria kansasii phagocytosis inhibition, we have examined in that context three series of peptides derived from the RGDVY and GRGD sequences. It was found that the levels of the inhibitory activity depend on the amino acid composition as well as on the particular peptide sequence. Distinct inhibitory activity was found in the case of thymopentin (RKDVY), the active fragment of thymopoietin. In this case the Mycobacterium phagocytosis inhibition should be combined with general immunostimulatory activity of RKDVY peptide. Our examination of a series of GRGDV analogs with a successively prolonged oligo-Gly linker inserted into the peptide chain showed that the distance between the Arg and Asp residues required for such an activity should be about 9A.—Authors’ Abstract


Mycobacteria are responsible for a number of human and animal diseases and are classical intracellular pathogens, living inside macrophages rather than as free-living organisms during infection. Numerous intracellular pathogens, including Listeria monocytogenes, Shigella flexneri, and Rickettsia rickettsii, exploit the host cytoskeleton by using actin-based motility for cell spread during infection. Here we show that Mycobacterium marinum, a natural pathogen of fish and frogs and an occasional pathogen of humans, is capable of actively inducing actin polymerization within macrophages. M. marinum that polymerized actin were free in the cytoplasm and propelled by actin-based motility into adjacent cells. Immunofluorescence demonstrated the presence of host cytoskeletal proteins, including the Arp2/3 complex and vasodilator-stimulated phosphoprotein, throughout the actin tails. In contrast, Wiskott-Aldrich syndrome protein localized exclusively at the actin-polymerizing pole of M. marinum. These findings show that M. marinum can escape into the cytoplasm of infected macrophages, where it can recruit host cell cytoskeletal factors to induce actin polymerization leading to direct cell to cell spread.—Authors’ Abstract


To investigate the role of innate immunity in variable efficacy of Mycobacterium bovis BCG vaccination in Malawi and the United Kingdom, we examined 24-hr tumor necrosis factor alpha, interleukin-1beta (IL-1beta), and IL-10 responses to mycobacterial purified protein derivatives (PPDs). The rank order in stimulatory potency for different PPDs was the same for all three cytokines. Before vaccination Malawians made higher pro- and anti-inflammatory responses than did United Kingdom subjects. Fewer than 5% of United Kingdom subjects made IL-10 in response to any PPD, compared to 19 to 57% responders among Malawians. Priming for regulatory IL-10 may contribute to the smaller increase in gamma interferon responses in Malawians.
compared to United Kingdom subjects following BCG vaccination.—Authors’ Abstract


Both CD4+ and CD8+ T cells from mice infected with Mycobacterium avium suffered a high rate of apoptosis, beginning with the onset of the immune response and culminating in the loss of T cells from the tissues and loss of IFN-gamma production. Fas expression increased over the course of infection on both T cell populations, as did their susceptibility to the induction of apoptosis in vitro by anti-Fas mAb. Nevertheless, although the rate of apoptosis among CD4+ T cells from infected mice was reduced to normal levels in lpr mice with a defective Fas, CD8+ T cells were unaffected, implying that Fas/FasL interaction was not important in these cells in vivo. Conversely, over-expression of B-cell lymphoma-2 (Bcl-2), which is known to protect T cells from apoptosis signalled through the TNF receptor or due to the withdrawal of cytokines, totally protected CD8+ T cells from infected mice but had no effect on CD4+. It is of interest that these two contrasting pathways of T-cell apoptosis operate at the same time during a single infection.—Authors’ Abstract

Immuno Pathology (Leprosy)


Protection against intracellular pathogens such as Mycobacterium leprae is critically dependent on the function of NK cells at early stages of the immune response and on Th1 cells at later stages. In the present report we evaluated the role of IL-18 and IL-13, two cytokines that can influence NK cell activity, in the generation of Mycobacterium leprae-derived hsp65-cytotoxic T lymphocytes (CTL) from peripheral blood mononuclear cells (PBMC) of leprosy patients. We demonstrated that IL-18 modulates hsp65-induced CTL generation and collaborates with IL-12 for this effect. In paucibacillary (PB) patients and normal controls (N) depletion of NK cells reduces the cytolytic activity. Under these conditions, IL-12 cannot up-regulate this CTL generation, while, in contrast, IL-18 increases the cytotoxic activity both in the presence or absence of NK cells. IL-13 down-regulates the hsp65-induced CTL generation and counteracts the positive effect of IL-18. The negative effect of IL-13 is observed in the early stages of the response, suggesting that this cytokine affects IFNgamma production by NK cells. mRNA coding for IFNgamma is induced by IL-18 and reduced in the presence of IL-13, when PBMC from N or PB patients are stimulated with hsp65. Neutralization of IL-13 in PBMC from multibacillary (MB) leprosy patients induces the production of IFNgamma protein by lymphocytes. A modulatory role on the generation of hsp65 induced CTL is demonstrated for IL-18 and IL-13 and this effect takes place through the production of IFNgamma.—Authors’ Abstract


In this presentation an attempt has been made to describe the nine-banded armadillo as an animal model, probably the only one in which lepromatous leprosy similar to that found in humans can be experimentally produced. Some unique features of the physiology of the animal are mentioned. The pathology and the microbiology of leprosy in the armadillo are described in detail. The discovery of lepromatous leprosy in the wild armadillos in the southern parts of the United States, the transmission of disease among
them through trauma and thorn pricks and the pathogenesis of the disease are presented. The impact of leprosy in the wild animals may have on human leprosy is discussed.—Indian Journal of Pathology and Microbiology


*See Current Literature, Experimental Infections, p. 235.*


We have investigated the expression of chemokines and their receptors in leprosy skin lesions using immunohistochemistry. Skin biopsies from 25 leprosy patients across the leprosy spectrum, 11 patients undergoing type I reversal reactions and four normal donors were immunostained by ABC peroxidase method using antibodies against CC and CXC chemokines and their receptors. Using an *in situ* hybridization technique we have also studied the expression of monocyte chemoattractant protein 1 (MCP-1), RANTES and interleukin (IL)-8 chemokines mRNA in leprosy skin lesions. Chemokines and receptor expression was detected in all leprosy skin biopsies. Expression of CC chemokines MCP-1 (p <0.01) and RANTES (p <0.01) were elevated significantly in borderline tuberculoid leprosy in reversal reaction compared to non-reactional borderline tuberculoid leprosy, but there was no difference in the expression of IL-8 chemokine. Surprisingly, there was no significant difference in the expression of mRNA for MCP-1, regulated upon activation normal T cell expressed and secreted (RANTES) and IL-8 chemokines. Here, the presence of a neutrophil chemoattractant IL-8 in leprosy lesions, which do not contain neutrophils, suggests strongly a role of IL-8 as a monocyte and lymphocyte recruiter in leprosy lesions. These results suggest that the chemokines and their receptors, which are known to chemoattract T lymphocytes and macrophages, are involved in assembling the cellular infiltrate found in lesions across the leprosy spectrum.—Authors’ Abstract


The functional status of adrenocortical hormones and their relationship to the pattern of inflammatory cytokines in the lepromatous and tuberculoid poles of leprosy were investigated. Interleukin (IL)-1beta, IL-6 and tumour necrosis factor (TNF)-alpha plasma levels, C-reactive protein (CRP) concentrations and erythrocyte sedimentation rates (ESR) were significantly higher in LL/BL (lepromatous) leprosy patients than in control subjects. There was a significant positive correlation between IL-6 and TNF-alpha plasma levels and ESR and CRP concentrations. IL-1beta was positively correlated with ESR but not with CRP. Both baseline and stimulated adrenocorticotropic hormone and cortisol plasma levels were not different between patients and control subjects. In contrast, adrenal androgen dehydroepiandrosterone sulphate (DHEA-S) plasma levels were significantly lower in leprosy patients than in sex-matched control subjects. There was a significant inverse correlation between DHEA-S and IL-6, TNF-alpha, and CRP concentrations. IL-1beta was positively correlated with ESR but not with CRP. Both baseline and stimulated adreno-corticotropic hormone and cortisol plasma levels were not different between patients and control subjects. In contrast, adrenal androgen dehydroepiandrosterone sulphate (DHEA-S) plasma levels were significantly lower in leprosy patients than in sex-matched control subjects. There was a significant inverse correlation between DHEA-S and IL-6, TNF-alpha, and CRP concentrations. This finding may be of pathogenetic significance in this disease and in other inflammatory states.—Authors’ Abstract


In leprosy, cell-mediated immunity (CMI) is more significant than humoral response to eliminate intracellular pathogen. T cell defect is a common feature in lepromatous leprosy (LL) patients as compared to tuberculoid type (TT) patients. For efficient initiation of CD4+, T cell response requires T cell receptor (TCR) activation and costimulation provided by molecules on antigen-presenting cells (APC) and their counter receptors on T cells. In our previous study, the defective T cell function in LL patients was restored to a proliferating state with the release of TH1 type cytokines using mycobacterial antigen(s) with two immunomodulators (Murabutide (MDP-BE) and T cell epitope of Trat protein of Escherichia coli) by presenting the antigen in particulate form in vitro to PBMC derived from leprosy patients. This observation prompted us to study the expression of the costimulatory molecules (CD80, CD86, CD28, CD152), other accessory molecules (TCR alphabeta/gammadelta) and T cell lineage molecules (CD4+ and CD8+) during constitutive and activated state of peripheral blood mononuclear cells (PBMC) derived from normal and leprosy individuals using different formulations of Mycobacterium leprae total cell wall antigen (MLCWA), Trat and MDP-BE using flow cytometric analysis. An increased surface expression of CD80, CD86 and CD28 but decreased CD152 expression was observed when PBMC of normal, BT/TT (tuberculoid) and BL/LL (lepromatous) patients were stimulated in vitro with MLCWA+ MDP-BE+ Trat peptide using liposomal mode of antigen delivery, while opposite results were obtained with the antigen alone. Antibody inhibition study using antihuman CD80 or CD86 completely abolished the T cell lymphoproliferation, thereby reconfirming the importance of these costimulatory molecules during T cell activation/differentiation. Though the liposome-entrapped antigen formulation has no effect on expression of alphabeta/gammadelta T cell receptor, the constitutive levels of TCR gammadelta were high in lepromatous patients. Thus, TCR bearing gammadelta appears to have a negligible regulatory role in peripheral blood of leprosy patients. The percentage of cells positive for CD4+ are increased in inducible state in all the three groups, while CD8+-positive cells were decreased in LL patients, thereby reconfirming the fact that priming of CD4+ cells are necessary for producing final effector functions. Lastly, intracellular cytokine staining experiment indicated that CD4+ cells are the major producers of IFN-gamma but not NK cells. The study highlights the reversal of T cell anergy especially in lepromatous patients through the modulation of costimulatory molecule expression under the influence of Th1 cytokines, i.e., IL-2 and IFNgamma.—Authors’ Abstract


New tools are urgently needed for the detection of latent tuberculosis (TB). We evaluated the diagnostic potential of 2 novel Mycobacterium tuberculosis complex-specific candidate antigens (Rv2653 and Rv2654) and investigated T cell recognition during natural infection in humans and experimental infection in guinea pigs. Peripheral blood mononuclear cells stimulated with peptide pools covering the full length of Rv2654 induced interferon-gamma release in 10 of 19 patients with TB. Neither Rv2654 single peptides nor Rv2654 pools were recognized by bacille Calmette-Guerin-vaccinated donors. However, peptides from Rv2653 were recognized by both patients group. The cross-reactive epitope(s) in Rv2653 were located in
a 36-amino acid stretch in the center of the molecule. Rv2654 also induced M. tuberculosis-specific skin-test responses in 3 of 4 aerosol-infected guinea pigs. Rv2654 is a strongly recognized T cell antigen that is highly specific for TB and has potential as a novel cell-mediated immunity-based TB diagnostic agent.—Authors’ Abstract


In recent years, there has been considerable focus on the discovery and characterization of proteins derived from Mycobacterium tuberculosis leading to the identification of a number of candidate antigens for use in vaccine development or for diagnostic purposes. Previous experiments have demonstrated an important immunological role for proteins encoded by the RD1 region, which is absent from all strains of bacillus Calmette-Guerin (BCG) but present in the genomes of virulent M. bovis and M. tuberculosis. Herein, we have studied human T-cell responses to the putative open reading frame (rv3878) of the RD1 region. Immunoblot analysis revealed that rv3878 was expressed and the native protein was designated TB27.4. Immunological evaluations demonstrate that TB27.4 elicits a prominent immune response in human tuberculosis patients with a dominant region in the C-terminal part of the molecule. In contrast, very limited responses were seen in M. bovis BCG-vaccinated donors. This study therefore emphasizes the diagnostic potential of proteins encoded by the RD1 region.—Authors’ Abstract


Transforming growth factor beta (TGF-beta) is a cytokine which has been shown to suppress the antimycobacterial immune responses of humans and experimental animals. In this study, the contributions of TGF-beta to cytokine production in vivo were investigated by using the established guinea pig model of tuberculous pleurisy. Mycobacterium bovis BCG-vaccinated guinea pigs were injected intrapleurally with heat-killed virulent Mycobacterium tuberculosis. Eight days following induction of an antigen-specific pleural effusion, guinea pigs were injected intrapleurally with anti-TGF-beta 1 or isotype control antibody. The following day, pleural exudates were removed, and the fluid volume and characteristics of the infiltrating cells were determined. Pleural fluid was analyzed for total interferon (IFN) and tumor necrosis factor (TNF) protein levels by using appropriate bioassays. RNA from pleural effusion cells was examined to determine TGF-beta 1, TNF-alpha, IFN-gamma, and interleukin-8 mRNA levels by using real-time PCR. Proliferative responses of pleural effusion lymphocytes were examined in response to concanavalin A and purified protein derivative (PPD) in vitro. Treatment with anti-TGF-beta 1 resulted in decreased pleural fluid volume and decreased cell numbers in the pleural space along with an increased percentage of lymphocytes and a decreased percentage of neutrophils. The bioactive TNF protein levels in pleural fluid were increased in guinea pigs treated with anti-TGF-beta 1, while the bioactive IFN protein concentrations were not altered. Expression of TGF-beta 1 and TNF-alpha mRNA was significantly increased following TGF-beta 1 neutralization. Finally, PPD-induced proliferative responses of pleural cells from anti-TGF-beta 1-treated animals were significantly enhanced. Thus, TGF-beta 1 may be involved in the resolution of this local, mycobacterial antigen-specific inflammatory response.—Authors’ Abstract


Animal models of tuberculosis point to a protective role for MHC class I-restricted CD8(+) T cells, yet it is unclear how these cells protect or whether such findings extend to humans. Here we report that macrophages infected with Mycobacterium tuberculosis, rapidly process and present an early secreted antigenic target (ESAT-6)-specific HLA class I-restricted CD8(+) T cell epitope. When cocultured with CD8(+) T cells restricted through classical HLA class I molecules the growth of bacilli within macrophages is significantly impaired after 7 days. This slow antimycobacterial activity did not correlate with macrophage lysis but required cell contact. We also found that inhibitors of apoptosis either had no effect or augmented the CD8-mediated suppressive activity, suggesting that an activation signal might be involved. Indeed we show that CD8(+) T cells were able to activate macrophages through receptors that include CD95 (Fas). Consistent with these findings the CD8-mediated suppression of mycobacterial growth was partially reversed by Fas blockade. These data identify a previously unrecognized CD8(+) T cell-mediated mechanism used to control an intracellular infection of macrophages.—Authors’ Abstract


Recognition of Mycobacterium tuberculosis by the innate immune system is essential in the development of an adaptive immune response. Mycobacterial cell wall components activate macrophages through Toll-like receptor (TLR) 2, suggesting that this innate immune receptor plays a role in the host response to M. tuberculosis infection. After aerosol infection with either 100 or 500 live mycobacteria, TLR2-deficient mice display reduced bacterial clearance, a defective granulomatous response, and develop chronic pneumonia. Analysis of pulmonary immune responses in TLR2-deficient mice after 500 mycobacterial aerosol challenge showed increased levels of interferon-gamma, tumor necrosis factor-alpha, and interleukin-12p40 as well as increased numbers of CD4(+) and CD8(+) cells. Furthermore, TLR2-deficient mice mounted elevated Ag-specific type 1 T-cell responses that were not protective because all deficient mice succumb to infection within 5 months. Taken together, the data suggests that TLR2 may function as a regulator of inflammation, and in its absence an exaggerated immune inflammatory response develops.—Authors’ Abstract


The Mycobacterium tuberculosis alternate sigma factor, SigF, is expressed during stationary growth phase and under stress conditions in vitro. To better understand the function of SigF we studied the phenotype of the M. tuberculosis DeltasigF mutant in vivo during mouse infection, tested the mutant as a vaccine in rabbits, and evaluated the mutant’s microarray expression profile in comparison with the wild type. In mice the growth rates of the DeltasigF mutant and wild-type strains were nearly identical during the first 8 weeks after infection. At 8 weeks, the DeltasigF mutant persisted in the lung, while the wild type continued growing through 20 weeks. Histopathological analy-
sis showed that both wild-type and mutant strains had similar degrees of interstitial and granulomatous inflammation during the first 12 weeks of infection. However, from 12 to 20 weeks the mutant strain showed smaller and fewer lesions and less inflammation in the lungs and spleen. Intradermal vaccination of rabbits with the *M. tuberculosis* DeltasigF strain, followed by aerosol challenge, resulted in fewer tubercles than did intradermal *M. bovis* BCG vaccination. Complete genomic microarray analysis revealed that 187 genes were relatively underexpressed in the absence of SigF in early stationary phase, 277 in late stationary phase, and only 38 genes in exponential growth phase. Numerous regulatory genes and those involved in cell envelope synthesis were down-regulated in the absence of SigF; moreover, the DeltasigF mutant strain lacked neutral red staining, suggesting a reduction in the expression of envelope-associated sulfolipids. Examination of 5′-untranslated sequences among the down-regulated genes revealed multiple instances of a putative SigF consensus recognition sequence: GGTTCX(18)GGGTAT. These results indicate that in the mouse the *M. tuberculosis* DeltasigF mutant strain persists in the lung but at lower bacterial burdens than wild type and is attenuated by histopathologic assessment. Microarray analysis has identified SigF-dependent genes and a putative SigF consensus recognition site.


Mycobacterial lipids comprise a heterogeneous group of molecules capable of inducing T cell responses in humans. To identify novel antigenic lipids and increase our understanding of lipid-mediated immune responses, we established a panel of T cell clones with different lipid specificities. Using this approach we characterized a novel lipid antigen belonging to the group of diacylated sulfoglycolipids purified from *Mycobacterium tuberculosis*. The structure of this sulfoglycolipid was identified as 2-palmitoyl or 2-stearoyl-3-hydroxyphthioceranoyl-2′-sulfate-alpha’-d-trehalose (Ac(2)SGL). Its immunogenicity is dependent on the presence of the sulfate group and of the two fatty acids. Ac(2)SGL is mainly presented by CD1b molecules after internalization in a cellular compartment with low pH. Ac(2)SGL-specific T cells release interferon gamma, efficiently recognize *M. tuberculosis*-infected cells, and kill intracellular bacteria. The presence of Ac(2)SGL-responsive T cells *in vivo* is strictly dependent on previous contact with *M. tuberculosis*, but independent from the development of clinically overt disease. These properties identify Ac(2)SGL as a promising candidate to be tested in novel vaccines against tuberculosis.—Authors’ Abstract


Tuberculosis leads to immune activation and increased human immunodeficiency virus type 1 (HIV-1) replication in the lung. However, *in vitro* models of mycobacterial infection of human macrophages do not fully reproduce these *in vivo* observations, suggesting that there are additional host factors. Surfactant protein A (SP-A) is an important mediator of innate immunity in the lung. SP-A levels were assayed in the human lung by using bronchoalveolar lavage (BAL). There was a threefold reduction in SP-A levels during tuberculosis only in the radiographically involved lung segments, and the levels returned to normal after 1 month of treatment. The SP-A levels were inversely correlated with the percentage of neutrophils in BAL fluid, suggesting that low SP-A levels were associated with increased inflammation in the lung. Differentiated THP-1 macrophages were used to test the effect of decreasing SP-A levels on immune function. In the absence of infection with *Mycobacterium tuberculosis*, SP-A at doses ranging from 5 to 0.01 micro g/ml
inhibited both interleukin-6 (IL-6) production and HIV-1 long terminal repeat (LTR) activity. In macrophages infected with *M. tuberculosis*, SP-A augmented both IL-6 production and HIV-1 LTR activity. To better understand the effect of SP-A, we measured expression of CAAT/enhancer binding protein beta (C/EBPbeta), a transcription factor central to the regulation of IL-6 and the HIV-1 LTR. In macrophages infected with *M. tuberculosis*, SP-A reduced expression of a dominant negative isoform of C/EBPbeta. These data suggest that SP-A has pleiotropic effects even at the low concentrations found in tuberculosis patients. This protein augments inflammation in the presence of infection and inhibits inflammation in uninfected macrophages, protecting uninvolved lung segments from the deleterious effects of inflammation.—Authors’ Abstract


Controversial results have been obtained in studies of the effect of *Mycobacterium tuberculosis* on human immunodeficiency virus type 1 (HIV-1) replication in cells of the macrophage lineage. In the present study, monocyte-derived macrophages (MDMs), previously incubated for 2 days with heat-inactivated *M. tuberculosis*, were infected with HIV-1. *M. tuberculosis* consistently inhibited viral replication, and a similar result also was observed in the presence of supernatants from *M. tuberculosis*-stimulated MDMs, which indicates that this effect was mediated by soluble factors. Although CCR5-binding chemokines were induced by *M. tuberculosis* stimulation, the results of neutralization experiments indicated that it is unlikely that they were responsible for viral suppression. Inhibition occurred mainly after viral entry (demonstrated by use of a vesicular stomatitis virus G-pseudotyped HIV-1 and by analysis of HIV-1 early and late reverse-transcription products). Therefore, *M. tuberculosis*-induced factors may inhibit *in vitro* HIV-1 replication in macrophages by affecting an early postentry step in the HIV-1 cycle.—Authors’ Abstract


The RD1 genomic region is present in virulent strains of *Mycobacterium tuberculosis* (MTB), missing from the vaccine strain *M. bovis* BCG, and its importance to virulence has been established experimentally. Based on *in silico* analysis, it has been suggested that RD1 may encode a novel secretion system, but the mechanism by which this region affects virulence is unknown. Here we examined mutants disrupted in five individual RD1 genes. Both *in vitro* and *in vivo*, each mutant displayed an attenuated phenotype very similar to a mutant missing the entire RD1 region. Genetic complementation of individual genes restored virulence. Attenuated mutants could multiply within THP-1 cells, but they were unable to spread to uninfected macrophages. We also examined export of two immunodominant RD1 proteins, CFP-10 and ESAT-6. Export of these proteins was greatly reduced or abolished in each attenuated mutant. Again, genetic complementation restored a wild-type phenotype. Our results indicate that RD1 genes work together to form a single virulence determinant, and argue that RD1 encodes a novel specialized secretion system that is required for pathogenesis of MTB.—Authors’ Abstract

The TB1-5 76C monoclonal antibody raised against a synthetic 60-mer peptide in the N-terminal part of the Mce1A mammalian cell entry protein of Mycobacterium tuberculosis has previously been shown to react with a linear epitope in the KRRITPKD region, residues 131–138 in Mce1A, and to cross-react with Mce1F. Six additional monoclonal antibodies raised against the same peptide were also shown to cross-react with Mce1F. Four of them reacted with a linear epitope in the same area, indicating that this area is immunodominant but showed distinct differences in fine specificity. Two monoclonal antibodies did not react with synthetic peptides from this region on the solid phase in enzyme-linked immunosorbent assay, indicating greater influence of conformation on reactivity. None of the monoclonal antibodies reacted with 14-mer synthetic peptides from the corresponding area in Mce2A, Mce3A, Mce4A, M. avium, M. smegmatis or M. leprae. The reaction pattern of the monoclonal antibodies was analysed in relation to our model of the Mce1A molecule (AK Das, et al. Biochem Biophys Res Commun 2003;302:442–7). The epitope is located on the surface of Mce1A, at the distal beta-strand-loop region in the beta-domain supporting its potential role in promoting uptake of M. tuberculosis in host cells.

Monoclonal antibody TB1-5 19C cross-reacted with glutathione S-transferase of Schistosoma japonicum containing a PKE triplet. Monoclonal antibody TB1-5 76C gave a major band at about 44 kDa in Western blotting of M. tuberculosis sonicate, whereas polyclonal rabbit anti-Mce1A peptide antibodies reacting with the extended TTPKNPTKRRITPKDVI area of Mce1A showed a distinct band above the 160 kDa molecular mass standard.—Authors’ Abstract


We have investigated the substance derived from Mycobacterium tuberculosis (Mtbc) that induces interleukin (IL)-12 production by alveolar macrophages (AMs) in vitro. The cytosol fraction of live Mtbc H37Rv induced IL-12 production by AMs in a dose-dependent manner. The addition of interferon-gamma (IFN-gamma) augmented IL-12 production. IL-12-inducing activity by AMs (termed as surely active keeping rescue antigen, SAKRA) was purified by gel filtration and ion exchange column chromatography, and the molecular weight of SAKRA was estimated by gel filtration to be more than 700 kDa. SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting of SAKRA using rabbit anti-SAKRA antibody suggested that SAKRA is composed with several low molecular weight proteins. Amino acids sequence analysis of several bands after SDS-PAGE suggested that SAKRA is a part of ribosomes. RT-PCR showed that SAKRA induced not only expression of IL-12 p40 mRNA, but expression of tumor necrosis factor (TNF)-alpha and inducible nitric oxide synthase (iNOS) mRNA at least 6 hr after stimulation, suggesting that SAKRA activates the bactericidal activity of macrophages. To investigate the potential use of SAKRA as a vaccine against tuberculosis, SAKRA was administered to BALB/c mouse that had been immunized with BCG for 18 months, and mouse were infected with Mtbc H37Rv via a respiratory route. Replication of Mtbc in lungs and spleens was examined 6 weeks after infection. Administration of SAKRA to BCG-vaccinated mice significantly reduced the numbers of Mtbc in lungs and spleens as compared with BCG-vaccinated control mice. Taken together, these results suggest that SAKRA is one of the Mtbc-derived immunomodulatory substances which induce IL-12 production during infection and also increases mycobactericidal activities of macrophages, and that SAKRA may be a promising new vaccine candidate against tuberculosis.

Both innate and adaptive immune systems contribute to host defense against infection with Mycobacterium tuberculosis. NK cells have been associated with early resistance against intracellular pathogens and are known to be potent producers of the cytokine IFN-gamma. In C57BL/6 mice infected by aerosol exposure with M. tuberculosis, NK cells increased in the lungs over the first 21 days of infection. Expansion of the NK cell subset was associated with increased expression of activation and maturation markers. In addition, NK cells isolated from the infected lungs were capable of producing IFN-gamma and became positive for perforin. In vivo depletion of NK cells using a lytic Ab had no influence on bacterial load within the lungs. These findings indicate that NK cells can become activated during the early response to pulmonary tuberculosis in the mouse model and are a source of IFN-gamma, but their removal does not substantially alter the expression of host resistance.—Authors’ Abstract

Kanaujia, G. V., Motzel, S., Garcia, M. A., Andersen, P., and Gennaro, M. L.


Protective immunity against pulmonary tuberculosis (TB) is characterized by the formation in the lungs of granulomas consisting of macrophages and activated T cells producing tumor necrosis factor alpha and gamma interferon, both required for the activation of the phagocytes. In 90% of immunocompetent humans, this response controls the infection. To understand why immunity fails in the other 10%, we studied the lungs of six patients who underwent surgery for incurable TB. Histologic examination of different lung lesions revealed heterogeneous morphology and distribution of acid-fast bacilli; only at the surface of cavi ties, i.e., in granulomas with a patent connection to the airways, were there numerous bacilli. The mutation profile of the isolates suggested that a single founder strain of Mycobacterium tuberculosis may undergo genetic changes during treatment, leading to acquisition of additional drug resistance independently in discrete physical locales. Additional drug resistance was preferentially observed at the cavity surface. Cytokine gene expression revealed that failure to control the bacilli was not associated with a generalized suppression of cellular immunity, since cytokine mRNA was up regulated in all lesions tested. Rather, a selective absence of CD4(+) and CD8(+) T cells was noted at the luminal surface of the cavity. Preventing direct T-cell-macrophage interactions at this site, probably allowing luminal phagocytes to remain permissive for bacillary growth. In contrast, in the perinecrotic zone of the granulomas, the two cell types colocalized and bacillary numbers were substantially lower, suggesting that in this microenvironment an efficient bacteriostatic or bactericidal phagocyte population was generated.—Authors’ Abstract

CD40(-/-) mice succumbed to low-dose aerosol infection with *M. tuberculosis* due to deficient IL-12 production leading to impaired priming of IFN-gamma T cell responses. In contrast, CD40L(-/-) mice were resistant to *M. tuberculosis*. This asymmetry in outcome of infection between the two knockout strains is likely due to the existence of an alternative ligand for CD40. Both in vitro *M. tuberculosis* infection and recombinant *M. tuberculosis* Hsp70 elicited IL-12 production from WT dendritic cells. This response was absent in both CD40(-/-) dendritic cells and CD40(-/-) mice, suggesting that *M. tuberculosis* Hsp70 serves as an alternative ligand for CD40 in vivo.—Authors’ Abstract


Here we describe the identification of a new CD8(+) T-cell epitope, the GYAGTLQLSL nonamer, shared by the TB10.3 and TB10.4 proteins of the *Mycobacterium tuberculosis* ESAT-6 family. Cytotoxic T cells from mycobacterium-infected mice efficiently recognized this epitope. GYAGTLQLSL-specific T-cell hybridomas, which were able to recognize *Mycobacterium bovis* BCG-infected macrophages, were generated and now allow investigation of mycobacterial antigen processing through the major histocompatibility complex class I pathway.—Authors’ Abstract


The devR-devS two-component system of *Mycobacterium tuberculosis* was identified earlier and partially characterized in our laboratory. A devR::kan mutant of *M. tuberculosis* was constructed by allelic exchange. The devR mutant strain showed reduced cell-to-cell adherence in comparison to the parental strain in laboratory culture media. This phenotype was reversed on complementation with a wild-type copy of devR. The devR mutant and parental strains grew at equivalent rates within human monocytes either in the absence or in the presence of lymphocytic cells. The expression of DevR was not modulated upon entry of *M. tuberculosis* into human monocytes. However, guinea pigs infected with the mutant strain showed a significant decrease in gross lesions in lung, liver and spleen; only mild pathological changes in liver and lung; and a nearly 3 log lower bacterial burden in spleen compared to guinea pigs infected with the parental strain. Our results suggest that DevR is required for virulence in guinea pigs but is not essential for entry, survival and multiplication of *M. tuberculosis* within human monocytes in vitro. The attenuation in virulence of the devR mutant in guinea pigs together with DevR-DevS being a bona fide signal transduction system indicates that DevR plays a critical and regulatory role in the adaptation and survival of *M. tuberculosis* within tissues.—Authors’ Abstract


A comprehensive analysis of culture supernatant (CSN) proteins of *Mycobacterium tuberculosis* H37Rv was accomplished by combination of two-dimensional electrophoresis (2-DE), mass spectrometry, and N-terminal sequencing by Edman degradation. Analytical 2-DE gels resolved approximately 1250 protein spots from CSN of *M. tuberculosis* H37Rv, 381 of which were identified by mass spectrometry and/or Edman degradation. This study revealed 137 different proteins, 42 of which had previously been described as secreted. Compar-
ative proteome analysis of CSN from virulent *M. tuberculosis* H37Rv and attenuated *Mycobacterium bovis* BCG Copenhagen identified 39 *M. tuberculosis*-specific spots containing 27 different proteins, representing candidate antigens for novel vaccines and diagnostics in tuberculosis. These included five proteins encoded by open reading frames absent from *M. bovis* BCG, e.g., early secretory antigen target (Esat6), as well as 22 novel differential proteins, such as acetyl-CoA C-acetyltransferase (Rv0243) and two putative Esat6-like proteins (Rv1198, Rv1793).—Authors’ Abstract


Mycobacteria synthesize mycothiol (MSH) as a low-molecular-weight thiol that protects against oxidative stress in a similar role to that of glutathione in many other species. The absence of MSH in mammals suggests that enzymes from its biosynthetic pathway in *Mycobacterium tuberculosis* could be useful targets for drug design. The gene for MshB (Rv1170), the enzyme that catalyses the second step in MSH biosynthesis in *M. tuberculosis*, has been cloned and the protein has been expressed in Escherichia coli both in native and SeMet-substituted forms and crystallized in two crystal forms. One of these, prepared in the presence of beta-octylglucoside as a key additive, is suitable for high-resolution X-ray structural analysis. The crystals are orthorhombic, with unit-cell parameters a = 71.69, b = 83.74, c = 95.65 Å, space group P2(1)2(1)2(1) and two molecules in the asymmetric unit. X-ray diffraction data to 1.9 Å resolution have been collected.—Authors’ Abstract


Production of the Th1 cytokine IFN-gamma by T cells is considered crucial for immunity against *Mycobacterium tuberculosis* infection. We evaluated IFN-gamma production in tuberculosis in the context of signaling molecules known to regulate Th1 cytokines. Two populations of patients who have active tuberculosis were identified, based on their T cell responses to the bacterium. High responder tuberculosis patients displayed significant *M. tuberculosis*-dependent T cell proliferation and IFN-gamma production, whereas low responder tuberculosis patients displayed weak or no T cell responses to *M. tuberculosis*. The expression of the signaling lymphocytic activation molecule (SLAM)-associated protein (SAP) on cells from tuberculosis patients was inversely correlated with IFN-gamma production in those individuals. Moreover, patients with a nonfunctional SAP gene displayed immune responses to *M. tuberculosis* similar to those of high responder tuberculosis patients. In contrast to SAP, T cell expression of SLAM was directly correlated with responsiveness to *M. tuberculosis* Ag. Our data suggest that expression of SAP interferes with Th1 responses whereas SLAM expression contributes to Th1 cytokine responses in tuberculosis. The study further suggests that SAP and SLAM might be focal points for therapeutic modulation of T cell cytokine responses in tuberculosis.—Authors’ Abstract


Cell-mediated immunity, leading to *Mycobacterium tuberculosis* (Mt)-constraining granuloma formation, is the major component of host defense against tuberculosis and is regulated by the balance of cytokines se-
creted mostly by mononuclear phagocytes and lymphocytes. To better understand the role of monocytes in the regulation of the immune response against pulmonary tuberculosis, we examined IL-10, IL-12 and TNF-alpha release by monocytes from healthy purified protein derivative (PPD) reactors and pulmonary tuberculosis patients with or without systemic reactions (e.g., fever, weight loss, asthenia). Our study shows that, probably as a result of in vivo priming by circulating antigens, monocytes from patients, especially those with systemic manifestations, have a biased ex vivo cytokine secretion, with high IL-10 and TNF-alpha but low IL-12, in contrast with PPD reactors. Higher spontaneous IL-10 and TNF-alpha release persisted when monocytes were co-cultured with autologous lymphocytes. Challenge of patients’ monocytes with a virulent Mtb strain led to a further enhancement of IL-10 and TNF-alpha, but not IL-12, in contrast with PPD reactors. Higher spontaneous IL-10 and TNF-alpha release persisted when monocytes were co-cultured with autologous lymphocytes. Challenge of patients’ monocytes with a virulent Mtb strain led to a further enhancement of IL-10 and TNF-alpha, but not IL-12, in contrast with PPD reactors. Higher spontaneous IL-10 and TNF-alpha release persisted when monocytes were co-cultured with autologous lymphocytes. Challenge of patients’ monocytes with a virulent Mtb strain led to a further enhancement of IL-10 and TNF-alpha, but not IL-12, in contrast with PPD reactors. Higher spontaneous IL-10 and TNF-alpha release persisted when monocytes were co-cultured with autologous lymphocytes. Challenge of patients’ monocytes with a virulent Mtb strain led to a further enhancement of IL-10 and TNF-alpha, but not IL-12, in contrast with PPD reactors.
be determined. Here, we provide evidence that the attenuation of a DIM-deficient strain takes place during the acute phase of infection in both lungs and spleen of mice, and that this attenuation results in part from the increased sensitivity of the mutant to the cidal activity of reactive nitrogen intermediates released by activated macrophages. We also show that the DIM-deficient mutant, the growth and survival of which were not impaired within resting macrophages and dendritic cells, induced these cells to secrete more tumour necrosis factor (TNF)-alpha and interleukin (IL)-6 than the wild-type strain. Although purified DIM molecules by themselves had no effect on the activation of macrophages and dendritic cells in vitro, we found that the proper localization of DIMs in the cell envelope of *M. tuberculosis* is critical to their biological effects. Thus, our findings suggest that DIM production contributes to the initial growth of *M. tuberculosis* by protecting it from the nitric oxide-dependent killing of macrophages and modulating the early immune response to infection.—Authors’ Abstract


Purified protein derivative (PPD) RT23-recalled T-cell receptor (TCR) Vβ expression was studied in the peripheral blood of 42 pulmonary tuberculosis patients and 44 healthy controls from southern India, a region where tuberculosis is endemic. Forty-eight-hour whole-blood cultures in the presence or absence of PPD-RT23 were set up, and at the end of the culture period total RNA was extracted and cDNA was synthesized. Expression of various TCR Vβ families was assessed by using family-specific primers. PPD-specific expression (usage) of TCR Vβ families 4, 6, 8 to 12, and 14 was found in more controls than patients. Among the responders (individuals who showed PPD-specific expression), endemic controls had significantly higher responses than the patients had for TCR Vβ families 2, 3, 7, 13, and 17. The majority of the patients did not show usage of most of the TCR Vβ families, and this was attributed to T-cell downregulation. A four-way nested classification analysis revealed that TCR Vβ family 1, 5, 9, 12, and 13 usage in the context of HLA class II high-risk alleles) (DRB1*1501, DRB1*08, and DQB1*0601) and *Mycobacterium bovis* BCG scar status were the determining factors in susceptibility and resistance to tuberculosis. The healthier status of controls was attributed to the wider usage of many TCR Vβ families readily recalled by PPD, while the disease status of the patients was attributed to TCR Vβ downregulation and the resultant T-cell (memory cell?) unresponsiveness. Host genetics (HLA status) and BCG vaccination (scar status) seem to play important roles in skewing the immune response in adult susceptibility to pulmonary tuberculosis through TCR Vβ usage.—Tropical Disease Bulletin


Classic studies of tuberculosis (TB) revealed morphologic evidence of considerable heterogeneity of macrophages (MOs), but the functional significance of this heterogeneity remains unknown. We have used newly available specific antibodies for selected membrane and secretory molecules to examine the phenotype of MOs in situ in a range of South African patients with TB, compared with sarcoidosis. Patients were human immunodeficiency virus-negative adults and children, and the examined biopsy specimens included lung and lymph nodes. Mature pulmonary MOs (alveolar, interstitial, epithelioid and multinucleated giant cells) selectively expressed scavenger receptor type A and a novel carboxypeptidase-like antigen called carboxypeptidase-related vitellogenin-like MO molecule (CPVL). CPVL did not display enhanced expression
in sarcoidosis, vs. TB patients, as observed with angiotensin-converting enzyme (ACE), a related molecule. Immunocytochemical studies with surfactant proteins (SP)-A and -D showed that type II alveolar cells expressed these collectins, as did MOs, possibly after binding of secreted proteins. Studies with an antibody specific for the C-terminus of fractalkine, a tethered CX3C chemokine, confirmed synthesis of this molecule by bronchiolar epithelial cells and occasional endothelial cells. These studies provide new marker antigens and extend previous studies on MO differentiation, activation and local interactions in chronic human granulomatous inflammation in the lung.—Authors’ Abstract


Old mice can express a transient early resistance to infection with M. tuberculosis that requires the presence of CD8 T cells within the lungs. Further characterization of those CD8 T cells within the aged lung established that the majority of CD8 T cells from old mice expressed the IL-15 receptor (CD122) in combination with bright expression of CD44 (CD44(hi)), and were capable of producing IFN-gamma after T cell receptor cross-linking. It has previously been described that CD8 CD44(hi) T cells proliferate in response to IFN-I, acting via IL-15, and therefore we determined whether IFN-I signaling could be a participant in the response of CD8 T cells within the lungs of old mice infected with M. tuberculosis. We demonstrate here that IFN-I signaling was required for the expansion of CD8 T cells within the aging lung in response to infection with M. tuberculosis, but that IFN-I signaling had no influence on the capacity of old mice to express early resistance to an infection with M. tuberculosis. Resident CD8 T cells were still however capable of producing IFN-gamma, which we demonstrate here to be critical in the expression of early resistance, suggesting that the expression of early resistance requires the participation, but not expansion, of the CD8 T cell pool within the aging lung.—Authors’ Abstract


Mycobacterium tuberculosis is a facultative intracellular pathogen that parasitizes macrophages by modulating properties of the Mycobacterium-containing phagosome. Mycobacterial phagosomes do not fuse with late endosomal/lysosomal organelles but retain access to early endosomal contents by an unknown mechanism. We have previously reported that mycobacterial phosphatidylinositol analog lipoarabinomannan (LAM) blocks a trans-Golgi network-to-phagosome phosphatidylinositol 3-kinase-dependent pathway. In this work, we extend our investigations of the effects of mycobacterial phosphoinositides on host membrane trafficking. We present data demonstrating that phosphatidylinositol mannoside (PIM) specifically stimulated homotypic fusion of early endosomes in an ATP-, cytosol-, and N-ethylmaleimide sensitive factor-dependent manner. The fusion showed absolute requirement for small Rab GTPases, and the stimulatory effect of PIM increased upon partial depletion of membrane Rabs with RabGDI. We found that stimulation of early endosomal fusion by PIM was higher when phosphatidylinositol 3-kinase was inhibited by wortmannin. PIM also stimulated in vitro fusion between model phagosomes and early endosomes. Finally, PIM displayed in vivo effects in macrophages by increasing accumulation of plasma membrane-endosomal syntaxin 4 and transferrin receptor on PIM-coated latex bead phagosomes. In addition, inhibition of phagosomal acidification was detected with PIM-coated beads. The effects of PIM, along with the previous action of LAM, suggest that M. tuberculosis has evolved a two-prong strategy to modify its intracellular niche: its products block acquisition of late endosomal/lysosomal constituents, while facilitating fusion with early
endosomal compartments.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 262.

Microbiology


Nonpigmented and late-pigmenting rapidly growing mycobacteria (RGM) are increasingly isolated in clinical microbiology laboratories. Their accurate identification remains problematic because classification is labor intensive work and because new taxa are not often incorporated into classification databases. Also, 16S rRNA gene sequence analysis underestimates RGM diversity and does not distinguish between all taxa. We determined the complete nucleotide sequence of the rpoB gene, which encodes the bacterial beta subunit of the RNA polymerase, for 20 RGM type strains. After using in-house software which analyzes and graphically represents variability stretches of 60 bp along the nucleotide sequence, our analysis focused on a 723-bp variable region exhibiting 83.9 to 97% interspecies similarity and 0 to 1.7% intraspecific divergence. Primer pair Myco-F-Myco-R was designed as a tool for both PCR amplification and sequencing of this region for molecular identification of RGM. This tool was used for identification of 63 RGM clinical isolates previously identified at the species level on the basis of phenotypic characteristics and by 16S rRNA gene sequence analysis. Of 63 clinical isolates, 59 (94%) exhibited <2% partial rpoB gene sequence divergence from the corresponding type strain; they belonged to three taxa related to M. mucogenicum, Mycobacterium smegmatis, and Mycobacterium porcinum. For M. abscessus and M. mucogenicum, this partial sequence yielded a high genetic heterogeneity within the clinical isolates. We conclude that molecular identification by analysis of the 723-bp rpoB sequence is a rapid and accurate tool for identification of RGM.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 256.


Polymerase chain reaction (PCR) has been widely used due to its high specificity, sensitivity, and rapid turn-around time. However, inhibitory factors may be co-extracted with the target nucleic acid that will hinder the performance of PCR. In this study, DNA extraction methods for Mycobacterium avium subsp. paratuberculosis were evaluated including rapid lysis, organic extraction, silica-based and magnetic particle-based (MagaZorb) technologies on bacterial cells, and spiked bovine feces. Efficiency of the extraction was determined by PCR end point titration with primers targeting the insertion sequence, IS900. Results of
the end point titrations are identical for bacterial cells and spiked feces. Inhibition was observed in PCR with DNA isolated from spiked feces, and a 1/100 dilution was able to alleviate this problem with DNA extracted by MagaZorb. A 1/1000 dilution was required for the other three methods. MagaZorb proved to be more efficient at removing inhibitory factors and required the least labor and completion time. Further evaluation is required for its utilization in other clinical specimens.—Authors’ Abstract


The synthesis of a panel of oligosaccharides containing C-5 arabinofuranosyl residues (9–20) is described. These compounds are of interest as potential inhibitors of the alpha-(1→5)-arabinosyltransferase involved in the assembly of mycobacterial cell-wall arabinan. In the series of compounds prepared, the 5-OH group on the nonreducing residue(s) is replaced, independently, with an amino, azido, fluoro, or methoxy functionality. The synthesis of the target compounds involved the preparation of a series of C-5 modified arabinofuranosyl thioglycosides (24–26) and their subsequent coupling to the appropriate acceptor species (21–23). Deprotection of the glycosylation products afforded the azido, fluoro, or methoxy analogs directly. The amino derivatives were obtained in one additional step by reduction of the azido compounds.—Authors’ Abstract


Mycobacterium ulcerans is an environmental organism which is responsible for the disease Buruli ulcer, a necrotizing skin disease emerging in west Africa. M. ul-
cerans produces the polyketide-derived macrolide mycolactone, which is required for the immunosuppression and tissue damage which characterizes Buruli ulcer. We have extracted lipids from the cell envelope and culture filtrate from 52 isolates of Mycobacterium species, analyzed them with thin-layer chromatography, and tested them in a murine fibroblast cell line (L929) cytoxicity assay to investigate whether these mycobacterial species produce mycolactone. For these studies chloroform-methanol (2:1, vol/vol) extracts were prepared from representative fast- and slow-growing mycobacterial species. Isolates tested included 16 uncharacterized, slow-growing, environmental mycobacterial species isolated from areas in which M. ulcerans infection is endemic. Although several strains of mycobacteria studied produced cytopathic lipids, none of these produced a phenotype on cultured cells consistent with that produced by mycolactone. Two mycobacterial species, M. scrofulaceum and M. kansasii, and eight of the environmental mycobacterial isolates contained cell-associated lipids cytopathic to fibroblasts at concentrations of 33 to 1000 microg/ml. In contrast, mycolactone produces cytoxicity at less than 2 ng/ml. Analysis of 16S rRNA sequences from the eight environmental isolates suggests that these are novel mycobacterial species. Results from these studies suggest that, although production of cytopathic lipids is relatively common among mycobacterial species, the production of mycolactone as a cell-associated or secreted molecule appears so far to be restricted to M. ulcerans.—Author’s Abstract


In this study, we aimed to evaluate the frequency of non-tuberculous mycobacteria (NTM) isolated from clinical specimens using Polymerase Chain Reaction-Restriction Enzyme Analysis (PCR-REA) and to investigate the patients who had clinically signif-
Significant NTM infections in our hospital through the five year period from May 1997 to June 2002. A total of 364 mycobacterial strains isolated from clinical specimens which gave positive growth index in the BACTEC 460 radiometric system in Hacettepe University Hospital Clinical Microbiology Laboratory were evaluated by PCR-REA and clinical data were obtained from the patient records. Three hundred and one of the strains (82.7%) were identified as \textit{Mycobacterium tuberculosis} and 63 (17.3%) were identified as nontuberculous mycobacteria. Seven (11.1%) of 63 NTM patients were regarded as having clinical mycobacteriosis. Chronic obstructive pulmonary disease and other pre-existing lung diseases were seen in 39 (61.9%) of the patients, 11 (17.5%) of the patients had chronic renal failure. Four (6.3%) and 9 (14.3%) of them had AIDS and carcinomas, respectively. PCR-REA was found to be a reliable method for typing of our mycobacterial isolates to the species level. These data may shed light on the epidemiology of the mycobacterial species and help to select a proper treatment regimen.—Authors’ Abstract


BACKGROUND: Molecular identification of Mycobacterium species has two primary advantages when compared to phenotypic identification: rapid turn-around time and improved accuracy. The information content of the 5′ end of the 16S ribosomal RNA gene (16S rDNA) is sufficient for identification of most bacterial species. However, reliable sequence-based identification is hampered by many faulty and some missing sequence entries in publicly accessible databases. METHODS: In order to establish an improved 16S rDNA sequence database for the identification of clinical and environmental isolates, we sequenced both strands of the 5′ end of 16S rDNA (\textit{Escherichia coli} positions 54 to 510) from 199 mycobacterial culture collection isolates. All validly described species (n = 89; up to March 21, 2000) and nearly all published sequence variants were included. If the 16S rDNA sequences were not discriminatory, the internal transcribed spacer (ITS) region sequences (n = 84) were also determined. RESULTS: Using 5′-16S rDNA sequencing a total of 64 different mycobacterial species (71.9%) could be identified. With the additional input of the ITS sequence, a further 16 species or subspecies could be differentiated. Only \textit{Mycobacterium tuberculosis} complex species, \textit{M. marinum}/\textit{M. ulcerans} and the \textit{M. avium} subspecies could not be differentiated using 5′-16S rDNA or ITS sequencing. A total of 77 culture collection strain sequences, exhibiting an overlap of at least 80% and identical by strain number to the isolates used in this study, were found in the GenBank. Comparing these with our sequences revealed that an average of 4.31 nucleotide differences (S.D. ± 0.57) were present. CONCLUSIONS: The data from this analysis show that it is possible to differentiate most mycobacterial species by sequence analysis of partial 16S rDNA. The high-quality sequences reported here, together with ancillary information (e.g., taxonomic, medical), are available in a public database, which is currently being expanded in the RIDOM project http://www.ridom-rdna.de), for similarity searches.—Authors’ Abstract


A simple immunochromatographic assay, Capilia TB, using anti-MPB64 monoclonal antibodies, is a kit for discriminating between the \textit{Mycobacterium tuberculosis} complex and mycobacteria other than tubercle bacilli. The sensitivity of the kit was estimated to be 99.2% (381 of 384 samples). The sequencing analysis revealed that all of the Capilia TB-negative isolates had mutations within the mbp64 gene, leading to the production of an incomplete protein as a result of a deletion of the C-terminal region of the protein.—Authors’ Abstract

*Mycobacterium ulcerans* causes Buruli ulcer, the third most prevalent mycobacterial infection of immunocompetent humans after tuberculosis and leprosy. Recent work has shown that the production by *M. ulcerans* of mycolactone, a novel polyketide, may partly explain the pathogenesis of Buruli ulcer. To search for the genetic basis of virulence in *M. ulcerans*, we took advantage of the close genetic relationship between *M. ulcerans* and *Mycobacterium marinum* by performing genomic suppressive subtractive hybridization of *M. ulcerans* with *M. marinum*. We identified several DNA fragments specific to *M. ulcerans*, in particular, a type I polyketide synthase locus with a highly repetitive modular arrangement. We postulate that this locus is responsible for the synthesis of mycolactone in *M. ulcerans*.—Authors’ Abstract


*Mycobacterium ulcerans* is the causative agent of Buruli ulcer, one of the most common mycobacterial diseases of humans. Recent studies have implicated aquatic insects in the transmission of this pathogen, but the contributions of other elements of the environment remain largely unknown. We report here that crude extracts from two green algae added to the BACTEC 7H12B culture medium halved the doubling time of *M. ulcerans* and promoted biofilm formation. Using the 7H12B medium, modified by the addition of the algal extract, and immunomagnetic separation, we also demonstrate that *M. ulcerans* is associated with aquatic plants in an area of the Ivory Coast where Buruli ulcer is endemic. Genotype analysis showed that plant-associated *M. ulcerans* had the same profile as isolates recovered in the same region from both aquatic insects and clinical specimens. These observations implicate aquatic plants as a reservoir of *M. ulcerans* and add a new potential link in the chain of transmission of *M. ulcerans* to humans.—Authors’ Abstract


All mycobacterial species, including pathogenic *Mycobacterium tuberculosis*, synthesize an abundant class of phosphatidylinositol mannosides (PIMs) that are essential for normal growth and viability. These glycolipids are important cell-wall and/or plasma-membrane components in their own right and can also be hyperglycosylated to form other wall components, such as lipomannan and lipoarabinomannan. We have investigated the steps involved in the biosynthesis of the major PIM species in a new *M. smegmatis* cell-free system. A number of apolar and polar PIM intermediates were labelled when this system was continuously labelled or pulse-chase-labelled with GDP-[3H]Man, and the glycan head groups and the acylation states of these species were determined by chemical and enzymic treatments and octyl-Sepharose chromatography respectively. These analyses showed that (1) the major apolar PIM species, acyl-PIM2, can be synthesized by at least two pathways that differ in the timing of the first acylation step, (2) early PIM intermediates containing a single mannose residue can be modified with two fatty acid residues, (3) formation of polar PIM species from acyl-PIM2 is amphotericin-sensitive, indicating that polyprenol phosphate-Man, rather than GDP-Man, is the donor for these reactions, (4) modification of acylated PIM4 with alpha1-2- or alpha1-6-linked mannose residues is probably the branch point in the biosyntheses of polar PIM and lipoarabinomannan respectively and (5) GDP strongly
inhibits the synthesis of early PIM intermediates and increases the turnover of poly-preneol phosphate-Man. These findings are incorporated into a revised pathway for mycobacterial PIM biosynthesis.—Authors’ Abstract


In order to evaluate the capacity of laser scanning cytometry (LSC) to detect acid-fast bacilli directly on clinical samples, a comparison between Kinyoun-stained smears analyzed under light microscopy and propidium iodide-auramine-stained smears analyzed by LSC was performed. The results were compared with those for culture on BACTEC MGIT 960. LSC is a new, reliable methodology to detect MYCOBACTERIA.—Authors’ Abstract


Mycolic acids are major and specific constituents of the cell envelope of Corynebacterineae, a suborder of bacterial species including several important human pathogens such as Mycobacterium tuberculosis, Mycobacterium leprae, or Corynebacterium diphtheriae. These long-chain fatty acids are involved in the unusual architecture and impermeability of the cell envelope of these bacteria. The condensase, the enzyme responsible for the final condensation step in mycolic acid biosynthesis, has remained an enigma for decades. By in silico analysis of various mycobacterial genomes, we identified a candidate enzyme, Pks13, that contains the four catalytic domains required for the condensation reaction. Orthologs of this enzyme were found in other Corynebacterineae species. A Corynebacterium glutami-


Hemolysin was quantified in 58 isolates of Mycobacterium avium from human, animal, and environmental sources. Human Mav-A and Mav-B isolates were the strongest producers; in contrast, animal and environmental Mav-A isolates and human, animal, and environmental Mav-C organisms were low-level producers. Hemolysin production was not restricted to isolates causing invasive infections.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 261.


Mycobacterium kansasii is one of the best known nontuberculous mycobacteria and large awareness exists about its involvement
in diseases both of immunocompetent and immunocompromised patients. Two phenotypic variants within this species, which differ for the virulence in guinea pig too, have been detected since 1962. It was however following recent progress in genetic studies that a large variability emerged. Major contributions to the disclosure of such findings came from the DNA probes hybridization, the nucleotide sequencing of 16 rDNA and internal transcribed spacer (ITS), and from the analyses of repetitive DNA sequences polymorphism. At present five subtypes of \textit{M. kansasii} are recognized, defined by the ITS sequence and by the polymorphism revealed by different restriction enzyme technologies. Such variants differ from the epidemiological point of view too, with type i being isolated from humans, type ii both from humans and environment, and types iii, iv and v, from the environment only. A revision of the present taxonomic status of \textit{M. kansasii} and its splitting into different species or subspecies seems nowadays necessary.—Authors’ Abstract


Tuberculosis is a worldwide health problem posing increasing threat with the spread of HIV infection and drug resistant \textit{Mycobacterium tuberculosis} strains. Consequently, control of this disease has become a significant challenge despite the availability of chemotherapy and BCG vaccine. Drug resistance for all first-line anti-tuberculosis agents and some second-line agents has been observed. Moreover, the occurrence of strains of \textit{M. tuberculosis} resistant to multiple anti-tuberculosis drugs is increasing. Mechanisms of action and resistance of major anti-tuberculosis drugs are reviewed. In addition, the phenotypic drug resistance such as dormant or persistent tubercle bacilli and its importance are also emphasized. In order to combat the threat of drug resistant tuberculosis and to more effectively control the disease, an understanding of the mechanisms underlying drug resistance is necessary. This knowledge could be used for the development of molecular tests for rapid detection of drug resistant bacilli and future anti-tuberculosis drugs.—Authors’ Abstract


Naturally occurring anti-HIV-1 agent (+)-calanolide A was found to be active against all of the strains of \textit{Mycobacterium tuberculosis} tested, including those resistant to the standard antitubercular drugs. Efficacy evaluations in macrophages revealed that (+)-calanolide A significantly inhibited intracellular replication of \textit{M. tuberculosis} H37Rv at concentrations below the MIC observed \textit{in vitro}. Preliminary mechanistic studies indicated that (+)-calanolide A rapidly inhibits RNA and DNA synthesis followed by an inhibition of protein synthesis. Compared with known inhibitors, this scenario is more similar to effects observed with rifampin, an inhibitor of RNA synthesis. Since (+)-calanolide A was active against a rifampin-resistant strain, it is believed that these two agents may involve different targets. (+)-Calanolide A and its related pyranocoumarins are the first class of compounds identified to possess antimycobacterial and antiretroviral activities, representing a new pharmacophore for anti-TB activity.—Authors’ Abstract

\textbf{Microbiology (Leprosy)}


The polymorphism of TTC repeats in \textit{Mycobacterium leprae} was examined using the bacilli obtained from residents in villages at North Maluku where \textit{M. leprae} infections are highly endemic (as well as from patients at North Sulawesi of Indonesia) to elucidate
the possible mode of leprosy transmission. TTC genotypes are stable for several generations of passages in nude mice footpads and, hence, are feasible for the genotyping of isolates and epidemiological analysis of leprosy transmission. It was found that bacilli with different TTC genotypes were distributed among residents at the same dwelling in villages in which leprosy is endemic and that some household contacts harbored bacilli with a different genotype from that harbored by the patient. Investigations of a father-and-son pair of patients indicated that infections of bacilli with 10 and 18 copies, respectively, had occurred. Genotypes of TTC repeats were found to differ between a son under treatment and two brothers. These results reveal the possibility that in addition to exposure via the presence of a leprosy patient with a multibacillary infection who was living with family members, there might have been some infectious sources to which the residents had been commonly exposed outside the dwellings. A limited discriminative capacity of the TTC polymorphism in the epidemiological analysis implies the need of searching other useful polymorphic loci for detailed subdivision of clinical isolates.—Authors’ Abstract

Microbiology (Tuberculosis)


Two-component systems are major regulatory systems for bacterial adaptation to environmental changes. During the infectious cycle of Mycobacterium tuberculosis, adaptation to an intracellular environment is critical for multiplication and survival of the micro-organism within the host. The M. tuberculosis prrA gene, encoding the regulator of the two-component system PrrA-PrrB, has been shown to be induced upon macrophage phagocytosis and to be transiently required for the early stages of macrophage infection. In order to study the mechanisms of regulation of the PrrA-PrrB two-component system, PrrA and the cytoplasmic part of the PrrB histidine kinase were produced and purified as hexahistidine-tagged recombinant proteins. Electrophoretic mobility shift assays indicated that PrrA specifically binds to the promoter of its own operon, with in-
creased affinity upon phosphorylation. Moreover, induction of fluorescence was observed after phagocytosis of a wild-type *M. tuberculosis* strain containing the gfp reporter gene under the control of the prrA-prrB promoter, while this induction was not seen in a prrA/B mutant strain containing the same construct. These results indicate that the early intracellular induction of prrA depends on the autoregulation of this two-component system.—Authors’ Abstract


*Mycobacterium tuberculosis*, a Gram-positive bacterium, encodes a secreted Dsb-like protein annotated as Mtb DsbE (Rv2878c, also known as MPT53). Because Dsb proteins in *Escherichia coli* and other bacteria seem to catalyze proper folding during protein secretion and because folding of secreted proteins is thought to be coupled to disulfide oxidoreduction, the function of Mtb DsbE may be to ensure that secreted proteins are in their correctly folded states. We have determined the crystal structure of Mtb DsbE to 1.1 Å resolution, which reveals a thioredoxin-like domain with a typical CXXC active site. These cysteines are in their reduced state. Biochemical characterization of Mtb DsbE reveals that this disulfide oxidoreductase is an oxidant, unlike Gram-negative bacteria DsbE proteins, which have been shown to be weak reductants. In addition, the pK(a) value of the active site, solvent-exposed cysteine is approximately 2 pH units lower than that of Gram-negative DsbE homologs. Finally, the reduced form of Mtb DsbE is more stable than the oxidized form, and Mtb DsbE is able to oxidatively fold hirudin. Structural and biochemical analysis implies that Mtb DsbE functions differently from Gram-negative DsbE homologs, and we discuss its possible functional role in the bacterium.—Authors’ Abstract


Human tuberculosis (TB) is caused by the bacillus *Mycobacterium tuberculosis*, a subspecies of the *M. tuberculosis* complex (MTC) of mycobacteria. Postgenomic dissection of the *M. tuberculosis* proteome is ongoing and critical to furthering our understanding of factors mediating *M. tuberculosis* pathobiology. Towards this end, a 32-kDa putative glyoxalase in the culture filtrate (CF) of growing *M. tuberculosis* (originally annotated as Rv0577 and hereafter designated CFP32) was identified, cloned, and characterized. The cfp32 gene is MTC restricted, and the gene product is expressed *ex vivo* as determined by the respective Southern and Western blot testing of an assortment of mycobacteria. Moreover, the cfp32 gene sequence is conserved within the MTC, as no polymorphisms were found in the tested cfp32 PCR products upon sequence analysis. Western blotting of *M. tuberculosis* subcellular fractions localized CFP32 predominantly to the CF and cytosolic compartments. Data to support the *in vivo* expression of CFP32 were provided by the serum recognition of recombinant CFP32 in 32% of TB patients by enzyme-linked immunosorbent assay (ELISA) as well as the direct detection of CFP32 by ELISA in the induced sputum samples from 56% of pulmonary TB patients. Of greatest interest was the observation that, per sample, sputum CFP32 levels (a potential indicator of increasing bacterial burden) correlated with levels of expression in sputum of interleukin-10 (an immunosuppressive cytokine and a putative contributing factor to disease progression) but not levels of gamma interferon (a key cytokine in the protective immune response in TB), as measured by ELISA. Combined, these data suggest that CFP32 serves a necessary biological function(s) in tubercle bacilli and may
contribute to the *M. tuberculosis* pathogenic mechanism. Overall, CFP32 is an attractive target for drug and vaccine design as well as new diagnostic strategies.—Authors’ Abstract


Drug resistance and virulence of *Mycobacterium tuberculosis* are in part related to the pathogen’s antioxidant defense systems. KatG(−) strains are resistant to the first line tuberculostatic isoniazid but need to compensate their catalase deficiency by alternative peroxidase systems to stay virulent. So far, only NADH-driven and AhpD-mediated hydroperoxide reduction by AhpC has been implicated as such virulence-determining mechanism. We here report on two novel pathways which underscore the importance of the thioredoxin system for antioxidant defense in *M. tuberculosis*: (i) NADPH-driven hydroperoxide reduction by AhpC that is mediated by thioredoxin reductase and thioredoxin C and (ii) hydroperoxide reduction by the atypical peroxiredoxin TPx that equally depends on thioredoxin reductase but can use both, thioredoxin B and C. Kinetic analyses with different hydroperoxides including peroxynitrite qualify the redox cascade comprising thioredoxin reductase, thioredoxin C, and TPx as the most efficient system to protect *M. tuberculosis* against oxidative and nitrosative stress in situ.—Authors’ Abstract


Erp (Exported Repetitive Protein), also known as P36, Pirg and Rv3810, is a member of a mycobacteria-specific family of extracellular proteins. In pathogenic species, the erp gene has been described as a virulence factor. The Erp proteins comprise three domains. The N- and C-terminal domains are similar in all mycobacterial species, while the central domain consists of a repeated module that differs considerably between species. Here we show that the Erp protein is loosely attached to the surface and that the carboxy-terminal domain, which displays hydrophobic features, anchors Erp at the surface of the bacillus. The hydrophobic region is not necessary for the complementation of the altered colony morphology of a *Mycobacterium smegmatis* erp- mutant but proved to be necessary to achieve resistance to detergent at wild-type levels.—Authors’ Abstract


In the present study we attempted to develop a PCR-based epidemiological tool for the differentiation of *Mycobacterium tuberculosis* isolates. Use of the designed primers Mtb1 (5′-CCG-GCG-GGG-CCG-GCG-G) and Mtb2 (5′-CGG-CGG-CAA-CGG-CGG-C) targeting frequently repeated 16-bp sequences in combination with primers sited at the inverted repeats flanking IS6110 allowed differentiation of *M. tuberculosis* isolates.—Authors’ Abstract


Ethionamide (ETH) is a structural analog of the antituberculosis drug isoniazid (INH). Both of these drugs target InhA, an enzyme involved in mycolic acid biosynthesis. INH requires catalase-peroxidase (KatG) activation, and mutations in katG are a major INH
Recently an enzyme (EthA) capable of activating ETH has been identified. We sequenced the entire ethA structural gene of 41 ETH-resistant Mycobacterium tuberculosis isolates. We also sequenced two regions of inhA and all or part of katG. The MICs of ETH and INH were determined in order to associate the mutations identified with a resistance phenotype. Fifteen isolates were found to possess ethA mutations, for all of which the ETH MICs were \( \geq 50 \) microg/ml. The ethA mutations were all different, previously unreported, and distributed throughout the gene. In eight of the isolates, a missense mutation in the inhA structural gene occurred. The ETH MICs for seven of the InhA mutants were \( \geq 100 \) microg/ml, and these isolates were also resistant to \( \geq 8 \) microg of INH per ml. Only a single point mutation in the inhA promoter was identified in 14 isolates. A katG mutation occurred in 15 isolates, for which the INH MICs for all but 1 were \( \geq 32 \) microg/ml. As expected, we found no association between katG mutation and the level of ETH resistance. Mutations within the ethA and inhA structural genes were associated with relatively high levels of ETH resistance. Approximately 76% of isolates resistant to \( \geq 50 \) microg of ETH per ml had such mutations.—Authors’ Abstract


The ability of Mycobacterium tuberculosis auxotrophs to survive long-term starvation was measured. Tryptophan and histidine auxotrophs did not survive single-amino-acid starvation, whereas a proline auxotroph did. All three auxotrophs survived complete starvation. THP-1 cells were also able to restrict the growth of the tryptophan and histidine auxotrophs.—Author’s Abstract


We previously used a mycobacteriophage L5-derived integrating vector to demonstrate that glnE and aroK are essential genes in Mycobacterium tuberculosis by showing that we were unable to excise the integrated vector when it carried the only functional copy of these genes. We tested three systems to replace the integrated copy with alternative alleles. The most efficient method was to transform the strain with a second copy of the integrating vector. Excision of the resident vector and integration of the incoming vector occurred at an extremely high efficiency. This technique will allow us to study the role and functionality of essential genes in this important human pathogen.—Authors’ Abstract

Shimono, N., Morici, L., Casali, N., Cantrell, S., Sidders, B., Ehrt, S., and Riley, L. W. Hypervirulent mutant of

Inositol is utilized by Mycobacterium tuberculosis in the production of its major thiol and of essential cell wall lipoglycans. We have constructed a mutant lacking the gene encoding inositol-1-phosphate synthase (ino1), which catalyses the first committed step in inositol synthesis. This mutant is only viable in the presence of extremely high levels of inositol. Mutant bacteria cultured in inositol-free medium for four weeks showed a reduction in levels of mycothiol, but phosphatidylinositol mannoside, lipomannan and lipoarabinoman-

An estimated one-third of the world’s population is latently infected with *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis. Here, we demonstrate that, unlike wild-type *M. tuberculosis*, a strain of *M. tuberculosis* disrupted in the mce1 operon was unable to enter a stable persistent state of infection in mouse lungs. Instead, the mutant continued to replicate and killed the mice more rapidly than did the wild-type strain. Histological examination of mouse lungs infected with the mutant strain revealed diffusely organized granulomas with aberrant inflammatory cell migration.

Murine macrophages infected *ex vivo* with the mutant strain were reduced in their ability to produce tumor necrosis factor alpha, IL-6, monocyte chemoattractant protein 1, and nitric oxide (NO), but not IL-4. The mce1 mutant strain complemented with the mce1 genes stimulated tumor necrosis factor alpha and NO production by murine macrophages at levels stimulated by the wild-type strain. These observations indicate that the mce1 operon mutant is unable to stimulate T helper 1-type immunity in mice. The hypervirulence of the mutant strain may have resulted from its inability to stimulate a proinflammatory response that would otherwise induce organized granuloma formation and control the infection without killing the organism. The mce1 operon of *M. tuberculosis* may be involved in modulating the host inflammatory response in such a way that the bacterium can enter a persistent state without being eliminated or causing disease in the host.—Authors’ Abstract


Capacity of certain *Mycobacterium tuberculosis* isolates to grow more rapidly in human macrophages may be indicative of increased virulence. Significant differences were observed in intracellular growth of two isolates from sites of tuberculosis transmission, with an outbreak-associated strain growing faster than a strain causing disease in only one person. Activated THP-1 cells are a suitable alternative to peripheral blood monocyte models.—Authors’ Abstract


Pathogenetic processes that facilitate the entry, replication, and persistence of *Mycobacterium tuberculosis* (MTB) in the mammalian host likely include the regulated expression of specific sets of genes at different stages of infection. Identification of genes that are differentially expressed *in vivo* would provide insights into host-pathogen interactions in tuberculosis (TB); this approach might be particularly valuable for the study of human TB, where experimental opportunities are limited. In this study, the levels of selected MTB mRNAs were quantified *in vitro* in axenic culture, *in vivo* in the lungs of mice, and in lung specimens obtained from TB patients with active disease. We report the differential expression of MTB mRNAs associated with iron limitation, alternative carbon metabolism, and cellular hypoxia, conditions that are thought to exist within the granulomatous lesions of TB, in the lungs of wild-type C57BL/6 mice as compared with bacteria grown *in vitro*. Analysis of the same set of mRNAs in lung specimens obtained from TB patients revealed differences in MTB gene expression in humans as compared with mice.—Authors’ Abstract

Mycobacterium tuberculosis possesses five genes with significant homology to the resuscitation-promoting factor (Rpf) of Micrococcus luteus. The M. luteus Rpf is a secreted approximately 16-kDa protein which restores active growth to cultures of M. luteus rendered dormant by prolonged incubation in stationary phase. More recently, the Rpf-like proteins of M. tuberculosis have been shown to stimulate the growth of extended-stationary-phase cultures of Mycobacterium bovis BCG. These data suggest that the Rpf proteins can influence the growth of mycobacteria; however, the studies do not demonstrate specific functions for the various members of this protein family, nor do they assess the function of M. tuberculosis Rpf homologues in vivo. To address these questions, we have disrupted each of the five rpf-like genes in M. tuberculosis Erdman, and analyzed the mutants for their growth in vitro and in vivo. In contrast to M. luteus, for which rpf is an essential gene, we find that all of the M. tuberculosis rpf deletion mutant strains are viable; in addition, all show growth kinetics similar to Erdman wild type both in vitro and in mouse organs following aerosol infection. Analysis of rpf expression in M. tuberculosis cultures from early log phase through late stationary phase indicates that expression of the rpf-like genes is growth phase-dependent, and that the expression patterns of the five M. tuberculosis rpf genes, while overlapping to various degrees, are not uniform. We also provide evidence that mycobacterial rpf genes are expressed in vivo in the lungs of mice acutely infected with virulent M. tuberculosis.—Authors’ Abstract


The mechanisms utilized by Mycobacterium tuberculosis to establish, maintain, or reactivate from latent infection in the host are largely unknown but likely include genes that mediate adaptation to conditions encountered during persistence. Previously, a two-component signal transduction system, mprAB, was found to be required in M. tuberculosis for establishment and maintenance of persistent infection in a tissue- and stage-specific fashion. To begin to characterize the role of this system in M. tuberculosis physiology and virulence, a functional analysis of the mprA and mprB gene products was initiated. Here, evidence is presented demonstrating that sensor kinase MprB and response regulator MprA function as an intact signal-transducing pair in vitro and in vivo. Sensor kinase MprB can be autophosphorylated, can donate phosphate to MprA, and can act as a phospho-MprA phosphatase in vitro. Correspondingly, response regulator MprA can accept phosphate from MprB or from small phosphodonor including acetyl phosphate. Mutagenesis of residues His249 in MprB and Asp48 in MprA abolished the ability of these proteins to be phosphorylated in vitro. Introduction of these alleles into Mycobacterium bovis BCG attenuated virulence in macrophages in vivo. Together, these results support a role for the mprAB two-component system in M. tuberculosis physiology and pathogenesis. Characterization of two-component signal transduction systems will enhance our understanding of processes regulated by M. tuberculosis during acute and/or persistent infection in the host.—Authors’ Abstract


The stress-induced extracytoplasmic sigma factor E (SigE) of Mycobacterium tuberculosis shows increased expression after heat shock, sodium dodecyl sulfate treatment, and oxidative stress, as well as after phagocytosis in macrophages. We report that deletion of sigE results in delayed
lethality in mice without a significant reduction of bacterial numbers in lungs.—Authors’ Abstract


We examined the time course (7 weeks) of thermal hyperalgesia and light touch allodynia in rats after intradermal administration of Mycobacterium butyricum. Nociceptive thresholds to heat and light touch were assessed. Paw edema and temperature, motor function, body weight, and proprioception were also tested. Some rats developed arthritis (named AA rats) but others did not (named non-AA rats). Both groups were compared with healthy animals. Persistent hyperalgesia was found in both groups; in AA rats it appeared before clinical evidence of arthritis. Transient allodynia occurred only after edema development and fell when edema decreased. Motor function was impaired only in AA rats. The results of this study demonstrate that hyperalgesia, but not allodynia, appeared after Mycobacterium butyricum in both groups, suggesting that changes in sensitivity were not merely the result of local hypersensitivity of the inflamed tissue, but may also be due to alterations in nociception in the central nervous system.—Authors’ Abstract


Pigs were immunised with recombinant BCG (rBCG) expressing a truncated form of GP5 (lacking the first 30 NH(2)-terminal residues) (rBCGGP5) and M protein (rBCGM) of porcine reproductive and respiratory syndrome virus (PRRSV). At 30 days post-inoculation (dpi), pigs inoculated with rBCGGP5 and rBCGM developed a specific humoral immune response against the viral proteins, as detected by commercial ELISA and Western blot tests, and at 60 dpi, three out of five animals developed neutralizing antibodies with titers ranging from 1:4 to 1:8. At 67 dpi, an IFN-gamma response against BCG antigens, but not against the viral proteins, was detected by ELISPOT in inoculated pigs. Following challenge with a pathogenic strain of PRRSV, pigs inoculated with rBCG showed lower (p < 0.05) temperature, viremia and virus load in bronchial lymph nodes than control animals, suggesting the establishment of partial protection against PRRSV infection.—Authors’ Abstract


Whether the intranasal (i.n.) route of Mycobacterium bovis BCG vaccination provides better protection against pulmonary tuberculosis than subcutaneous (s.c.) vaccination remains an incompletely solved issue. In the present study, we compared both immune responses and protection elicited by single BCG vaccinations via the i.n. or s.c. route in BALB/c mice. While both i.n. and s.c. vaccination triggered comparable levels of primary immune activation in the spleen and draining lymph nodes, i.n. vaccination led to a greater antigen-specific gamma interferon recall response in splenocytes than s.c. vaccination upon secondary respiratory mycobacterial challenge, accompanied by an increased frequency of antigen-specific lymphocytes. There was also a quicker cellular response in the lungs of i.n. vaccinated mice upon mycobacterial challenge. Mice vaccinated i.n. were found to be much better protected, particularly in the lung, than s.c. vaccinated counterparts against pulmonary tuberculosis at both 3 and 6 months postvaccination. These results suggest that the i.n. route of vaccination improves the protective effect of the current BCG vaccine.—Authors’ Abstract
Chung, S. W., Choi, S. H., and Kim, T. S.  

Interferon-gamma (IFN-gamma) is closely associated with the generation of cell-mediated immunity and resistance to intracellular parasites. Interleukin-18 (IL-18) is known to strongly induce IFN-gamma production by T cells and natural killer (NK) cells. To determine whether the paracrine secretion of IL-18 can efficiently stimulate the resistance to Mycobacterium avium complex (MAC) infection, 3T3 fibroblasts were stably transfected to secrete bioactive IL-18 and their effects on MAC infection were investigated in genetically susceptible BALB/c mice, compared with that of free recombinant IL-18. Immunization with IL-18-secreting fibroblasts (3T3/IL-18) during intranasal infection with MAC resulted in a significant decrease in bacterial load of lung during the entire 8-week observation period, while rIL-18 reduced the bacterial load at initial 1 week but not by 8 weeks postinfection. Immunization with the 3T3/IL-18 cells induced and maintained significantly higher levels of cytotoxic activity and nitric oxide production by lung cells than those of rIL-18 immunization. Furthermore, lung cells in mice injected with the 3T3/IL-18 cells showed persistent production of IFN-gamma throughout the 8-week period, while rIL-18 reduced the bacterial load at initial 1 week but not by 8 weeks postinfection. Immunization with the 3T3/IL-18 cells induced and maintained significantly higher levels of cytotoxic activity and nitric oxide production by lung cells than those of rIL-18 immunization. Furthermore, lung cells in mice injected with the 3T3/IL-18 cells showed persistent production of IFN-gamma throughout the 8-week period, suggesting that the 3T3/IL-18 cells induced the resistance to MAC infection via IFN-gamma production. This work suggests that IL-18-secreting fibroblasts may serve as a vehicle for paracrine secretion of IL-18 in immunotherapy of MAC infection.—Authors’ Abstract


The Mycobacterium tuberculosis complex includes Mycobacterium bovis, which causes tuberculosis in most mammals, including humans. In previous work, it was shown that M. bovis ATCC 35721 has a mutation in its principal sigma factor gene, sigA, causing a single amino acid change affecting binding of SigA with the accessory transcription factor WhiB3. ATCC 35721 is avirulent when inoculated subcutaneously into guinea pigs but can be restored to virulence by integration of wild-type sigA to produce M. bovis WAg320. Subsequently, it was surprising to discover that WAg320 was not virulent when inoculated intratracheally into the Australian brushtail possum (Trichosurus vulpecula), a marsupial that is normally very susceptible to infection with M. bovis. In this study, an in vivo complementation approach was used with ATCC 35721 to produce M. bovis WAg322, which was virulent in possums, and to identify the virulence-restoring gene, phoT. There are two point deletions in the phoT gene of ATCC 35721 causing frameshift inactivation, one of which is also in the phoT of BCG. Knockout of phoT from ATCC 35723, a virulent strain of M. bovis, produced M. bovis WAg758, which was avirulent in both guinea pigs and possums, confirming that phoT is a virulence gene. The effect on virulence of mode of infection versus animal species susceptibility was investigated by inoculating all the above strains by aerosol into guinea pigs and mice and comparing these to the earlier results. Characterization of PhoT indicated that it plays a role in phosphate uptake at low phosphate concentrations. At least in vitro, this role requires the presence of a wild-type sigA gene and appears separate from the ability of phoT to restore virulence to ATCC 35721. This study shows the advantages of using different animal models as tools for the molecular biological investigation of tuberculosis virulence.—Authors’ Abstract

Collins, D. M., Kawakami, R. P., Wards, B. J., Campbell, S., and de Lisle, G. W.  

SETTING: Molecular techniques are now available to develop new live tuberculosis
vaccines by producing avirulent strains of the *Mycobacterium tuberculosis* complex with known genes deleted. **OBJECTIVES:** Determine if removal of esat-6 from new live tuberculosis vaccines with known attenuating mutations affects their vaccine efficacy and if it could enable the development of discriminating diagnostic tests. **DESIGN:** Remove the esat-6 gene by allelic exchange from two illegitimate mutants of *Mycobacterium bovis* that had previously been shown to have similar vaccine efficacy to BCG in a guinea pig vaccination model. Determine the effect this removal has on virulence, vaccine efficacy and skin test reactivity in guinea pigs. **RESULTS:** Two double knockout strains of *M. bovis* were produced and their virulence and vaccine efficacy were compared to their parent strains. Removal of the esat-6 gene had no significant effect on vaccine efficacy. In skin tests, animals inoculated with the double knockout strains reacted to PPD but not ESAT-6, whereas those inoculated with the parent strains had similar skin test reactivity to both PPD and esat-6. **CONCLUSION:** Removal of esat-6 from new live tuberculosis vaccine candidates has no significant effect on vaccine properties but does enable the use of skin tests to distinguish between vaccination and infection.—Authors’ Abstract


Tuberculosis (TB) is the most common opportunistic disease and a potentially fatal complication among immunocompromised individuals infected with human immunodeficiency virus (HIV). Effective vaccination against TB in persons with HIV has been considered unlikely because of the central role that CD4 cells play in controlling tuberculous infections. Here we show that the vaccination of CD8(−/−) mice with a TB DNA vaccine cocktail did not significantly enhance protective responses to a *Mycobacterium tuberculosis* infection. In contrast, immunization with a DNA vaccine cocktail or with the current TB vaccine, *Mycobacterium bovis* BCG, induced considerable antituberculosis protective immunity in immune-deficient mice lacking CD4 cells. In vaccinated CD4(−/−) animals, substantially reduced bacterial burdens in organs and much improved lung pathology were seen 1 month after an aeroenic *M. tuberculosis* challenge. Importantly, the postchallenge mean times to death of vaccinated CD4(−/−) mice were significantly extended (mean with DNA cocktail, 172 ± 7 days; mean with BCG, 156 ± 22 days) compared to that of naive CD4(−/−) mice (33 ± 6 days). Furthermore, the treatment of DNA-vaccinated CD4(−/−) mice with an anti-CD8 or anti-gamma interferon (IFN-gamma) antibody significantly reduced the effect of immunization, and neither IFN-gamma(−/−) nor tumor necrosis factor receptor-deficient mice were protected by DNA immunization; therefore, the primary vaccine-induced protective mechanism in these immune-deficient mice likely involves the secretion of cytokines from activated CD8 cells. The substantial CD8-mediated protective immunity that was generated in the absence of CD4 cells suggests that it may be possible to develop effective TB vaccines for use in HIV-infected populations.—Authors’ Abstract


Tuberculoid (TT) and lepromatous leprosy (LL) develop in the human host depending on his ability to trigger a specific cellular immune response(CIR). Different genes have been demonstrated in susceptibility/protection and may explain the forms of leprosy. The major histocompatibility complex (MHC) play an important role. The aim of the study was to explore the contribution of human leukocyte antigen (HLA) DRB1, DQA1, DQB1 and DQ promoter genes in LL Mexican patients. Six families (26 LL, three TT patients and 27 controls)
were analyzed; 114 unrelated patients were compared with 204 controls. Class I typing was done by the standard microlymphocytotoxicity and class II typing using PCR-SSOP. Haplotype segregation correlated with specific CIR in vivo and in vitro using lepromin. Haplotype sharing was significantly deviated in the affected sibs \((p = 0.01)\). Six healthy sibs were non-responders to lepromin and four of them were DQ1 homozygotes. DQ1 was significantly associated with LL and with non-responders. We set up macrophage activation experiments after infecting these cells with \(5 \times 10^6\) bacilli to demonstrate if elimination occurred in the context or DQ1. When DQ1 was present on macrophages and on T cells, bacteria were poorly eliminated from the cell \((32\%)\) while when absent, 76\% of the individuals were able to eliminate the bacilli \((p = 0.03)\). DRB1*1501 DQA1*0102-DQB1*0602 (DQ1 subtype) was significantly increased in the patients, indicating its participation in susceptibility. QBP 5.11/5.12 promoter present in the mentioned haplotype, and QAP 1.4, linked to DRB1*1301/02 haplotypes were also associated. Two mechanisms are suggested: the promoter polymorphisms may influence allele expression and thus the amount of peptides presented to the T-cell receptor, leading to a deficient CIR: HLA restriction is important for vaccine design; the way peptides anchor the DRB1*1501 groove may be relevant to the activation of TH1 cells, which contribute to an efficient presentation of peptides inducing a protective T-cell response.—Authors’ Abstract

**Holten-Andersen, L., Doherty, T. M., Korsholm, K. S., and Andersen, P.**


Recombinant, immunodominant antigens derived from *Mycobacterium tuberculosis* can be used to effectively vaccinate against subsequent infection. However, the efficacy of these recombinant proteins is dependent on the adjuvant used for their delivery. This problem affects many potential vaccines, not just those for tuberculosis, so the discovery of adjuvants that can promote the development of cell-mediated immunity is of great interest. We have previously shown that the combination of the cationic surfactant dimethyl dioctadecyl ammonium bromide and the immunomodulator modified lipid A synergistically potentiates Th1 T-cell responses. Here we report a screening program for other adjuvants with reported Th1-promoting activity and identify a second novel adjuvant formulation that drives the development of Th1 responses with an extremely high efficacy. The combination of dimethyl dioctadecyl ammonium bromide and the synthetic cord factor trehalose dibehenate promotes strong protective immune responses, without overt toxicity, against *M. tuberculosis* infection in a vaccination model and thus appears to be a very promising candidate for the development of human vaccines.—Authors’ Abstract

**Hsieh, M. J., Junqueira-Kipnis, A. P., Hoeffner, A., Turner, O. C., and Orme, I. M.**


Vaccines which offer better protection than BCG are now badly needed for controlling tuberculosis infection throughout the world. Immunological adjuvants capable of inducing a TH1 type of protective response are necessary to augment the immune response, particularly in the case of subunit vaccines. It is now well established that oligodeoxynucleotides (ODN) containing cytidine phosphate guanosine (CpG) motifs enhance cell-mediated responses in *vivo* by increasing the production of the TH1 cytokines IL-12 and interferon gamma (IFNgamma). To determine if this would improve subunit vaccination of mice CpG ODN were added to a subunit vaccine consisting of the culture filtrate proteins (CFP) of *Mycobacterium tuberculosis* H37Rv. It was observed that although adding CpG ODN to the vaccines promoted substantially increased IFNgamma production by lymph node cells draining sites of inoculation, this failed to translate after aerosol challenge.
into any degree of enhancement of bacterial clearance in the lungs, influx of IFN-positive T cells, or changes in histopathology. These data suggest that the vaccine enhancing effects of CpG ODN are relatively transient.—Authors’ Abstract


A single sub-cutaneous injection of a plasmid DNA encoding a mycobacterial heat shock protein 65 (Hsp65) entrapped in biodegradable poly(lactic-co-glycolic acid) microspheres produced high titers of antibodies, measured 5 months after the injection in BALB/c mice. Splenocytes secreted IFN-gamma and exerted an anti-bacterial effect on macrophages infected in vitro with *Mycobacterium tuberculosis*. The results are encouraging with regard to obtaining good compliance and vaccination coverage with candidate plasmid DNA vaccines, especially in developing countries.—Authors’ Abstract


Analysis of T-cell subsets accumulating in the lungs of C57BL/6 mice chronically infected with *Mycobacterium tuberculosis* revealed that both CD4 and CD8 T-cell populations expressed a cell surface phenotype consistent with that of effector T cells and that a significant proportion of these cells were in the process of secreting gamma interferon.—Authors’ Abstract


Tuberculosis is one of the most economically devastating, zoonotic infections of captive non-human primates. The limitations of the tuberculin skin test, which is currently used to diagnose tuberculosis in living non-human primates, make it necessary to find new, simple, and economical diagnostic methods. We describe use of an enzyme-linked immunoassay to detect IgG antibodies against early secretory antigenic target (ESAT)-6, a small protein secreted by virulent tubercle bacilli, in paired (pre- and post-outbreak) sera from 57 non-human primates involved in an outbreak of *Mycobacterium bovis* infection in a research colony. Of 25 animals with tuberculosis lesions at necropsy, 22 (88%) had high serum levels of the ESAT-6 antibody. The ESAT-6 antibody was found in 16% (5/32) of post-outbreak sera from animals in which tuberculosis could not be confirmed at necropsy. The
strong association between the ESAT-6 antibody and tuberculosis in non-human primates documented in this study, together with the robustness of the serologic assay, make the ESAT-6 ELISA a valuable tool for diagnosis of tuberculosis in captive non-human primates.—Authors’ Abstract


Tuberculosis, a bacterial disease prevalent since ancient times, continues to cause the most deaths globally compared with all other diseases. The causative agent *Mycobacterium tuberculosis* is responsible for different types of tuberculosis in humans; however, pulmonary tuberculosis is the most common and causes the most deaths. *Mycobacterium tuberculosis* is an intracellular pathogenic bacterium, which has developed sophisticated mechanisms to survive inside host mononuclear phagocytes and thus evade the host immune system. This is attributed primarily to an inadequate immune response toward infecting bacteria, which results in temporary growth inhibition rather than death and subsequently allows the bacteria to multiply immensely, leading to full-blown disease in an individual. This disease has become a challenge due to poor diagnosis, a low-efficiency tuberculosis vaccine (*Mycobacterium bovis* Bacillus Calmette-Guerin [BCG]), a long-term antibacterial chemotherapy regimen (approximately 6 months), and an emergence of multiple drug resistant strains of *Mycobacterium tuberculosis* especially in people with human immune deficiency virus (HIV) infection, for whom researchers worldwide must develop effective short-term chemotherapy and an effective vaccine. In this review different aspects of vaccines in tuberculosis are discussed, and these include the traditional BCG vaccine, the modern auxotrophic vaccine, the subunit or acellular vaccine; and a DNA vaccine. We discuss also the potential of mycobacterial lipids as a vaccine or as an adjuvant in the future. Since complete genome information of *Mycobacterium tuberculosis* H37Rv and bioinformatics tools are available, it is possible to develop new strategies for a better and effective tuberculosis vaccine, which can replace the traditional BCG vaccine.—Authors’ Abstract


In this study, we focused on three leukocyte-rich guinea pig cell populations, bronchoalveolar lavage (BAL) cells, resident peritoneal cells (PC), and splenocytes (SPC). BAL cells, SPC, and PC were stimulated either with live attenuated *Mycobacterium tuberculosis* H37Ra or with live or heat-killed virulent *M. tuberculosis* H37Rv (multiplicity of infection of 1:100). Each cell population was determined to proliferate in response to heat-killed virulent H37Rv, whereas no measurable proliferative response could be detected upon stimulation with live mycobacteria. Additionally, this proliferative capacity (in SPC and PC populations) was significantly enhanced upon prior vaccination with *Mycobacterium bovis* BCG. Accordingly, in a parallel set of experiments we found a strong positive correlation between production of antigen-specific bioactive tumor necrosis factor alpha (TNF-alpha) and prior vaccination with BCG. A nonspecific stimulus, lipopolysaccharide, failed to induce this effect on BAL cells, SPC, and PC. These results showed that production of bioactive TNF-alpha from mycobacterium-stimulated guinea pig cell cultures positively correlates with the vaccination status of the host and with the virulence of the mycobacterial strain.—Authors’ Abstract

Guinea pig eosinophils were positively identified in bronchoalveolar lavage populations and in the lung granulomas of *Mycobacterium tuberculosis*-infected guinea pigs. It is possible that the rapid influx of these cells, and their subsequent degranulation during acute pulmonary tuberculosis, may play a key role in the susceptibility of this animal model.—Authors’ Abstract


*Mycobacterium bovis* bacillus Calmette-Guerin (BCG) vaccination is efficacious for newborns or adults with no previous exposure to environmental mycobacteria. To determine the relative contribution and the nature of gammadelta T-cell receptor-positive T cells in newborns, compared to CD4(+) T cells, in immunity induced by *M. bovis* BCG vaccination, 4-week-old specific-pathogen-free pigs were vaccinated with *M. bovis* BCG and monitored by following the gammadelta T-cell immune responses. A flow cytometry-based proliferation assay and intracellular staining for gamma interferon (IFN-gamma) were used to examine gammadelta T-cell responses. Pigs were found to mount Th1-like responses to *M. bovis* BCG vaccination as determined by immunoproliferation and IFN-gamma production. The gammadelta T-cell lymphoproliferation and IFN-gamma production to stimulation with mycobacterial antigens were significantly enhanced by *M. bovis* BCG vaccination. The relative number of proliferating gammadelta T cells after stimulating peripheral blood mononuclear cells with *Mycobacterium tuberculosis* H37Rv culture filtrate protein was higher than that of CD4(+) T cells at an early time point after *M. bovis* BCG vaccination, but CD4(+) T cells were found to be more abundant at a later time point. Although the gammadelta T-cell responses were dependent on the presence of CD4(+) T cells for the cytokine interleukin-2, the enhanced gammadelta T cells were due to the intrinsic changes of gammadelta T cells caused by *M. bovis* BCG vaccination rather than being due solely to help from CD4(+) T cells. Our study shows that gammadelta T cells from pigs at early ages are functionally enhanced by *M. bovis* BCG vaccination and suggests an important role for this T-cell subset in acquired immunity conferred by *M. bovis* BCG vaccination.—Authors’ Abstract


A DNA vaccine codifying the mycobacterial hsp65 can prevent infection with *Mycobacterium tuberculosis* in a prophylactic setting and also therapeutically reduce the number of bacteria in infected mice. The protective mechanism is thought to be related to Th1-mediated events that result in bacterial killing. To determine the best method of hsp65 introduction for vaccination efficacy against tuberculosis (TB), we evaluated the immunogenicity and protection of DNA-hsp65 administered by gene gun bombardment or intramuscular (i.m.) injection of naked DNA. Immunization by gene gun induced immune response with plasmid doses 100-fold lower than those required for intramuscular immunization. However, in contrast to intramuscular immunization, which was protective in these studies, gene gun immunization did not protect BALB/c mice against challenge infection.—Authors’ Abstract


To develop a murine model of paucibacillary tuberculosis for experimental chemotherapy of latent tuberculosis infection, mice were immunized with viable *Mycobacterium bovis* BCG by the aerosol or intravenous route and then challenged six weeks later with virulent *Mycobacterium tu-
berculosis. The day after immunization, the counts were 3.71 ± 0.10 log(10) CFU in the lungs of aerosol-immunized mice and 3.65 ± 0.11 and 4.93 ± 0.07 log(10) CFU in the lungs and spleens of intravenously immunized mice, respectively. Six weeks later, the lungs of all BCG-immunized mice had many gross lung lesions and splenomegaly; the counts were 5.97 ± 0.14 and 3.54 ± 0.07 log(10) CFU in the lungs and spleens of aerosol-immunized mice, respectively, and 4.36 ± 0.28 and 5.12 ± 0.23 log(10) CFU in the lungs and spleens of intravenously immunized mice, respectively. Mice were then aerosol challenged with *M. tuberculosis* by implanting 2.37 ± 0.13 log(10) CFU in the lungs. Six weeks after challenge, *M. tuberculosis* had multiplied so that the counts were 6.41 ± 0.27 and 4.44 ± 0.14 log(10) CFU in the lungs and spleens of control mice, respectively. Multiplication of *M. tuberculosis* was greatly limited in BCG-immunized mice. Six weeks after challenge, the counts were 4.76 ± 0.24 and 3.73 ± 0.34 log(10) CFU in the lungs of intravenously immunized and aerosol-immunized mice, respectively. In contrast to intravenously immunized mice, there was no detectable dissemination to the spleen in aerosol-immunized mice. Therefore, immunization of mice with BCG by the aerosol route prior to challenge with a low dose of *M. tuberculosis* resulted in improved containment of infection and a stable paucibacillary infection. This model may prove to be useful for evaluation of new treatments for latent tuberculosis infection in humans.

Pinto, R., Saunders, B. M., Camacho, L. R., Britton, W. J., Gicquel, B., and Triccas, J. A. *Mycobacterium tuberculosis* defective in phthiocerol dimydocerosate translocation provides greater protective immunity against tuberculosis than the existing bacille Calmette-Guerin (BCG), and this improved protective efficacy was maintained for at least 24 weeks after vaccination. Protection afforded by this attenuated strain coincided with a number of factors that were not associated with BCG vaccination: long-term persistence of the strain within the host, sustained and potent induction of antimycobacterial interferon-gamma-secreting cells equal to that induced by virulent *M. tuberculosis*, and elicitation of T cells recognizing dominant *M. tuberculosis* antigens absent from BCG. These results suggest that the BCG vaccine may be too attenuated to afford effective protective immunity against tuberculosis, and vaccine strains that can provide sustained delivery of mycobacterial antigens are promising antituberculosis vaccine candidates.—Authors’ Abstract


*Mycobacterium ulcerans* disease, or Buruli ulcer (BU), causes significant morbidity in West Africa. Clinically, the disease presents in the skin as either nonulcerative or ulcerative forms and often invades bones either subjacent to the skin lesion (contiguous osteomyelitis) or remote from the skin lesion (metastatic osteomyelitis). Osteomyelitis represents a severe form of the disease that often requires numerous surgical interventions, even amputations. Surgery is accepted as the present definitive treatment for BU. In the absence of an effective drug treatment, the need for the development of preventive and control strategies becomes paramount. No specific vaccine, however, is presently available for BU. Of 372 consecutive patients in Benin presenting with BU (confirmed by microbiological and histopathological analyses) whose *Mycobacterium bovis* BCG scar statuses were known, 196 children (<15 years old) and 108 adults had neonatal BCG vaccination scars. Of 196 children with BCG scars, 17 (8.7%) had os-
teomyelitis, while 7 of 28 children without BCG scars (25.0%) had osteomyelitis. Of 108 adults with BCG scars, 17 (15.7%) had osteomyelitis, while 14 of 40 adults without BCG scars (35.0%) had osteomyelitis. Our results show that effective BCG vaccination at birth provides significant protection against the development of *M. ulcerans* osteomyelitis in children and adults. Therefore, health authorities should give attention to the enhancement of neonatal BCG vaccination coverage in all countries of Africa where BU is endemic. Protection against severe forms of BU and childhood tuberculosis would likewise be improved by this intervention.—Authors’ Abstract


BACKGROUND & OBJECTIVES: In recent years the efficacy of BCG vaccine against tuberculosis has been questioned and there is no alternative vaccine available. Several strategies are being applied to get a satisfactory vaccine. Two approaches are generally considered: the subunit vaccines and the whole cell vaccines. The objective of this investigation was to evaluate an avirulent mycobacteria, *Mycobacterium habana*, as a whole cell vaccine to protect mice from infection of *M. tuberculosis* H37Rv. METHODS: AKR and immunocompromised SJL/J mice were immunized with live *M. habana* vaccine. These mice were challenged with *M. tuberculosis* H37Rv eight weeks later along with unimmunized control mice. Protection by *M. habana* vaccine was measured through several parameters, which included survival of challenged mice, dissemination of challenge strain and histopathology of lung tissues. RESULTS: *M. habana* vaccinated animals were healthier than the unvaccinated mice after challenge with *M. tuberculosis* and survived with significant increase in mean survival time. The viable count of challenge strain was at least 100-fold less in vaccinated mice than the control mice. The lung tissues in unvaccinated mice showed marked bronchopneumonia with clusters of acid fast bacilli, whereas vaccinated mice showed small areas of damage and evidence of protection subsequently. INTERPRETATION & CONCLUSION: It may be concluded from the evidence presented here that mice vaccinated with *M. habana* were protected from challenge with *M. tuberculosis* in both normal and immunocompromised states.—Authors’ Abstract


Tuberculosis is characterized by granuloma formation and caseous necrosis, but the factors causing tissue destruction are poorly understood. Matrix metalloproteinase (MMP)-9 (92-kDa gelatinase) secretion from monocytes is stimulated by *Mycobacterium tuberculosis* (*M. tb*) and associated with local tissue injury in tuberculosis patients. We demonstrate strong immunohistochemical MMP-9 staining in monocytic cells at the center of granuloma and adjacent to caseous necrosis in *M. tb*-infected patient lymph nodes. Minimal tissue inhibitor of MMPs-1 staining indicated that MMP-9 activity is unopposed. Because granulomas characteristically contain few mycobacteria, we investigated whether monocyte-monocyte cytokine networks amplify MMP-9 secretion. Conditioned medium from *M. tb*-infected primary human monocytes or THP-1 cells (CoMTB) stimulated MMP-9 gene expression and a >10-fold increase in MMP-9 secretion by monocytes at 3–4 days (p <0.009, vs controls). Although CoMTB stimulated dose-dependent MMP-9 secretion, MMP-1 (52-kDa collagenase) was not induced. Anti-TNF-alpha Ab but not IL-1R antagonist pretreatment decreased CoMTB-induced MMP-9 secretion by 50% (p = 0.0001). Anti-TNF-alpha Ab also inhibited MMP-9 secretion from monocytes by 50%, 24 hr after direct M. tb infection (p = 0.0002). Conversely, TNF-alpha directly stimulated dose-dependent MMP-9 secretion. Pertussis toxin inhibited CoMTB-
induced MMP-9 secretion and enhanced the inhibitory effect of anti-TNF-alpha Ab (p = 0.05). Although chemokines bind to G protein-linked receptors, CXCL8, CXCL10, CCL2, and CCL5 did not stimulate monocyte MMP-9 secretion. However, the response to cholera toxin confirmed that G protein signaling pathways were intact. In summary, MMP-9 within tuberculous granuloma is associated with tissue destruction, and TNF-alpha, critical for antimycobacterial granuloma formation, is a key autocrine and paracrine regulator of MMP-9 secretion.—Authors’ Abstract


Mycobacterium tuberculosis, the causative organism of tuberculosis, encounters oxidative stress during phagocytosis by the macrophage and following macrophage activation during an acquired immune response, and also from internally generated sources of radical oxygen intermediates through intermediary metabolism. We have identified the SenX3 protein, a sensor in 1 of the 11 complete pairs of two-component signal transduction systems in M. tuberculosis, as a possible orthologue of the Mak2p protein from the fission yeast Schizosaccharomyces pombe that is known to sense peroxide stress. Moreover, the SenX3-RegX3 two-component system was the top scoring hit in a homology search with the Escherichia coli ArcB-ArcA global control system of aerobic genes. Using structural modelling techniques we have determined that SenX3 contains a PAS-like domain found in a variety of prokaryotic and eukaryotic sensors of oxygen and redox. Mutants with knockouts of senX3 or of the accompanying transcriptional regulator regX3 were constructed and found to have reduced virulence in a mouse model of tuberculosis infection, the mutant bacteria persisting for up to 4 months post-infection; complemented mutants had regained virulence confirming that it was mutations of this two-component system that were responsible for the avirulent phenotype. This work identifies the PAS domain as a possible drug target for tuberculosis and mutations in the senX3-regX signal transduction system as potentially useful components of live vaccine strains.—Authors’ Abstract


Although various members of the pattern recognition Toll-like receptor (TLR) family have been implicated in host resistance to Mycobacterium tuberculosis infection, it remains unclear if the TLR4 receptor plays an important role. We demonstrate here that infection of TLR4-competent and TLR4-deficient mice on the C3H inbred mouse strain background had similar outcomes, measured in terms of the course of the disease, cell accumulation patterns in the lungs, and lung histopathology. These data argue against a significant role for TLR4 in immunity to tuberculosis in the mouse model.—Authors’ Abstract


OBJECTIVE: There is epidemiological and experimental evidence that exposure to mycobacteria has the potential to suppress the development of atopy and/or asthma. Delipidated, deglycolipidated and arabinogalactan-depleted autoclaved Mycobacterium vaccae (delipidated acid-treated M. vaccae) has been shown to suppress allergen-induced airway eosinophilia in mice. METHODOLOGY: Thirty-seven adults with stable moderately severe asthma who were skin prick test-positive to house dust mite were randomized to receive two doses 2 weeks apart of delipidated acid-treated M. vaccae (first dose 0.4 mg and second dose 0.8 mg) or
phosphate buffered saline, given as drops intranasally. Safety, tolerability and markers of asthma severity (including peak flow, FEV1, major and minor exacerbations, symptom scores and beta-agonist use), and nasal symptom scores, blood eosinophil and IgE levels were monitored for 8 weeks. RESULTS: Delipidated acid-treated M. vaccae was safe and well tolerated although there was an occasional mild local reaction. There were no statistically significant differences between the treatment group and placebo for any of the outcome variables. CONCLUSIONS: There is a requirement to elucidate the reasons why mycobacterial-based vaccines have not shown equivalent efficacy in human trials compared with animal models. The role of factors such as duration of disease, route of administration and the active component of mycobacteria need to be addressed.—Authors’ Abstract


Protein tyrosine kinases and tyrosine phosphatases from several bacterial pathogens have been shown to act as virulence factors by modulating the phosphorylation and dephosphorylation of host proteins. The identification and characterization of two tyrosine phosphatases namely MptpA and MptpB from Mycobacterium tuberculosis has been reported earlier. MptpB is secreted by M. tuberculosis into extracellular milieu and exhibits a pH optimum of 5.6, similar to the pH of the lysosomal compartment of the cell. To determine the role of MptpB in the pathogenesis of M. tuberculosis, we constructed a mptpB mutant strain by homologous recombination and compared the ability of parent and the mutant strain to survive intracellularly. We show that disruption of the mptpB gene impairs the ability of the mutant strain to survive in activated macrophages and guinea pigs but not in resting macrophages suggesting the importance of its role in the host-pathogen interaction. Infection of guinea pigs with the mutant strain resulted in a 70-fold reduction in the bacillary load of spleens in infected animals as compared with the bacillary load in animals infected with the parental strain. Upon reintroduction of the mptpB gene into the mutant strain, the complemented strain was able to establish infection and survive in guinea pigs at rates comparable to the parental strain. These observations demonstrate a role of MptpB in the pathogenesis of M. tuberculosis.—Authors’ Abstract


All the trials of immunotherapy of tuberculosis with killed Mycobacterium vaccae, published or not, that are known to the authors are reviewed here. Following an introduction giving a brief account of some earlier immunotherapies for tuberculosis, the origins of the concept of immunotherapy with M. vaccae are considered. Progress is traced from the early work with irradiation-killed organisms in leprosy to the study in London of modulation of tuberculin skin-test responses, and the first comparative trials in The Gambia and Kuwait. In the last of these studies, dosages and different preparations were compared. As a result of this subsequent studies have used 109 heat-killed organisms, equivalent to 1mg wet-weight of bacilli, as a standard dose. A series of small trials in Argentina, India, Nigeria, Romania, South Africa and Vietnam have pioneered the way forward, disclosing geographic variability, with South Africa as the only country where almost no effects were recorded. Together the studies have shown that a single dose may not be sufficient. These studies have confirmed the mode of action of M. vaccae to be regulation of cell-mediated immunity with enhancement of Th1 and down-regulation of Th2, and they have shown benefits in faster bacteriological conversion, reduction in ESR, recovery of body weight and resolution of radiological opacities, leading to better recovery from the disease even when given to patients receiving directly observed therapy, short-
course (DOTS). Three major randomised, placebo-controlled and partly blinded trials have been carried out in Africa. The first, in South Africa showed no \( M. \text{ vaccae} \)-related effects. The second trial, in Uganda, confirmed the observations made in the earlier studies of faster sputum conversion and better radiological clearance. The third trial, in Zambia and Malawi, showed a trend towards benefits in the treatment of HIV seronegative patients but failed to show beneficial effects in HIV seropositive patients. Studies in patients with multi-drug-resistant tuberculosis have shown that multiple doses of immunotherapy are required in most cases, and that these markedly improve cure-rates for these patients. This is especially so when they are also treated with chemotherapy tailored to the resistance pattern of their infecting organisms. A small study has just commenced in which repeated doses of \( M. \text{ vaccae} \) are being administered to a group of patients who have failed treatment with DOTS-Plus (directly observed therapy with drugs selected on the basis of drug susceptibility profiles). Late in the investigation came publications from China supporting and confirming the data in both drug-sensitive and drug-resistant disease, by the use of multiple injections of their own different preparation of \( M. \text{ vaccae} \). The trial that is now beginning in Vietnam of 3 doses of \( M. \text{ vaccae} \) in the treatment of newly diagnosed pulmonary tuberculosis, is accompanied by a chemotherapeutic regimen with a shortened continuation phase. If this important study is successful, immunotherapy with killed \( M. \text{ vaccae} \) should be introduced into the treatment regimens for tuberculosis worldwide.


MyD88 is an adaptor protein that plays a major role in TLR/IL-1 receptor family signaling. To understand the role of MyD88 in the development of murine tuberculosis \textit{in vivo}, MyD88 knockout (KO) mice aerially were infected with \textit{Mycobacterium tuberculosis}. Infected MyD88 mice were not highly susceptible to \textit{M. tuberculosis} infection, but they developed granulomatous pulmonary lesions with neutrophil infiltration which were larger than those in wild-type (WT) mice (p <0.01). The pulmonary tissue levels of mRNA for iNOS and IL-18 were slightly lower, but levels of mRNA for IL-1 beta, IL-2, IL-4, IL-6, IL-10, IFN-gamma, and TGF-beta were higher in MyD88 KO mice. IFN-gamma, TNF-alpha, IL-1 beta, and IL-12 also were high in the sera of MyD88 KO mice. There were no statistically significant differences in the expression of TNF-alpha, IL-12, and ICAM-1 mRNA between MyD88 KO and WT mice. Thus, MyD88 deficiency did not influence the development of murine tuberculosis. NF-kappa B activity was similar in the alveolar macrophages from the lung tissues of MyD88 KO and WT mice. Also, there may be a TLR2-specific, MyD88-independent IL-1 receptor/TLR-mediated pathway to activate NF-kappa B in the host defense against mycobacterial infection.—Author’s Abstract


OBJECTIVES: Profiles of host innate resistance to \textit{Mycobacterium fortuitum} (MFT) infection in mice and the roles of macrophages (Mphis) and NK cells in host resistance to MFT infection were studied. METHODS: MFT-infected mice with or without the treatments to reduce Mphis and NK cells were examined for survival and the bacterial loads in the kidneys during the course of infection. RESULTS: A unique profile of strain difference was found in the innate resistance of mice to MFT. A/J, C3H/He and DBA/2 mice were susceptible, while BALB/c, B10A and C57BL/6 mice were resistant, in terms of survival after MFT infection. Such profiles of host resistance to MFT were essentially correlated with the ability of individual strain mice to prevent the bacterial growth in the early periods after infection. These profiles were different from the strain difference controlled by Bcg gene. Studies using carrageenan, anti-asialo GM1 antibody, and NK cell-

SUMMARY: PURPOSE OF REVIEW: To summarize and evaluate critically recent progress with mycobacteria as a potential novel disease modifying treatment strategy in asthma. RECENT FINDINGS: The link between exposure to pathogenic or saprophytic mycobacteria and protection from allergic diseases is still controversial, and recent epidemiological studies, which addressed only exposure to Mycobacterium tuberculosis or bacillus Calmette-Guerin, did not help to clarify this issue. Moreover, the clear efficacy of mycobacterial treatment seen in animal models has not been reproduced in human asthma, and a recent small study testing the hypothesis that heat-killed Mycobacterium vaccae attenuates asthmatic reactions after allergen challenge did not provide convincing results. However, it has been shown that treatment of mice with M. vaccae induces the generation of allergen-specific T regulatory cells capable of suppressing allergen-mediated eosinophilic lung inflammation, suggesting that a general deficiency of T regulatory cell activity might be responsible for the increased prevalence of asthma. This hypothesis is supported by findings that a lack of T regulatory cells, as found in genetic disorders of man and mouse attributable to a mutation of Foxp3, a transcription factor specifically expressed by T regulatory cells, is associated with manifestations of severe atopy and autoimmunity, precisely the spectrum of diseases linked to the hygiene hypothesis. SUMMARY: Further studies on the relationship between mycobacteria and atopic disorders are needed, but there is reason to believe that the novel findings and molecular mechanisms associated with mycobacterial infections will further strengthen the currently unproved therapeutic value of immunotherapy with mycobacteria.—Authors’ Abstract


Vitamin D deficiency is associated with an increased risk for tuberculosis infection. Studies using in vitro systems indicate that 1,25-dihydroxyvitamin D(3) [i.e., 1,25(OH)(2)D(3)], the most active form of the vitamin, enhances mycobacterial killing by increasing nitric oxide (NO) production. To evaluate concurrently the role of 1,25(OH)(2)D(3) and NO on the host response to tuberculosis infection, mice deficient in NO synthase 2 (NOS2(−/−)) and/or vitamin D were aerosol-challenged with Mycobacterium bovis and subsequently evaluated for mycobacterial colonization and lesion formation. Infected NOS2(−/−) mice developed severe necrotizing pyogranulomatous inflammation of the lungs with heavy M. bovis colonization and systemic dissemination of the bacillus. Colonization and lung lesion area of NOS2(−/−) mice exceeded that of NOS2(+/+) mice. Additionally, disease progression was more rapid in NOS2(−/−) mice than in NOS2(+/+) mice. Lung colonization and lesion area of vitamin D deficient mice exceeded that of vitamin D replete mice, regardless of NOS2 phenotype. However, effects of vitamin D on colonization, but not lesion area, were more pronounced in NOS2(+/+) mice than in NOS2(−/−) mice. These findings are consistent with the current hypothesis that 1,25(OH)(2)D(3) enhances mycobacterial killing through a NO-dependent mechanism. As responses of NOS2(−/−) mice were affected by 1,25(OH)(2)D(3) deficiency, albeit to a lesser extent than were those of NOS2(+/+) mice, NO-independent actions of 1,25(OH)(2)D(3) also likely exist.—Authors’ Abstract
A study was conducted to investigate the influence of BCG vaccination or revaccination on tuberculin skin test reactivity, in order to guide the correct interpretation of this test in a setting of high neonatal BCG vaccination coverage and an increasing BCG revaccination coverage at school age. In 1997, we conducted tuberculin skin testing and BCG scar reading in 1148 children aged 7–14 years old in the city of Salvador, Bahia, Brazil. We measured the positive effect of the presence of one or two BCG scars on the proportion of tuberculin skin test results above different cut-off levels (induration sizes of ≥5 mm, ≥10 mm, and ≥15 mm) and also using several ranges of induration size (0, 1–4, 5–9, 10–14, and ≥15 mm). We also measured the effects that age, gender, and the school where the child was enrolled had on these proportions. The proportion of tuberculin results ≥10 mm was 14.2% (95% confidence interval (CI) = 8.0%–20.3%) for children with no BCG scar, 21.3% (95% CI = 18.5%–24.1%) for children with one BCG scar, and 45.0% (95% CI = 32.0%–58.0%) for children with two BCG scars. There was evidence for an increasing positive effect of the presence of one and two BCG scars on the proportion of results ≥5 mm and ≥10 mm. Similarly, there was evidence for an increasing positive effect of the presence of one and two scars on the proportion of tuberculin skin test results in the ranges of 5–9 mm and of 10–14 mm. The BCG scar effect on the proportion of results ≥5 mm and ≥10 mm did not vary with age. There was no evidence for BCG effect on the results ≥15 mm. In Brazilian school children, BCG-induced tuberculin reactivity is indistinguishable, for results under 15 mm, from reactivity induced by Mycobacterium tuberculosis infection. BCG revaccination at school age increases the degree of BCG-induced tuberculin reactivity found among school children. This information should be taken into account in tuberculin skin test surveys intended to estimate M. tuberculosis prevalence or to assess transmission patterns as well as in tuberculin skin testing of individuals used as an auxiliary tool in diagnosing tuberculosis. Taking this information into consideration is especially important when there is increasing BCG revaccination coverage.

The objectives in this epidemiology review are to measure and report the extent of morbidity and mortality due to tuberculosis (TB), the proportion of new sputum smear positive cases in districts and the status of cohort analysis as of 1999. As for leprosy, the main objective is to determine morbidity and the treatment outcomes of Multiple Drug Therapy (MDT). Based on the results obtained, a comprehensive action plan for prevention, control and monitoring of tuberculosis and leprosy cases and patients is being produced and implemented throughout the state. The analysis concentrated on patients diagnosed at all out-patient units and admitted in all of the state’s hospitals. The patient particulars were recorded using a standardized format based on TB and Leprosy Health Management Information System (TB HMIS). TB was the second highest by notification of communicable diseases in Malaysia in 2001. 29% or about one-third of the national TB cases are from Sabah. However, it has been noted that there was an average decline of 2.6% in annual notification since 10 years ago to date. There was also a reduction of 11.4% in 2001 as compared to annual notification in 2000. Immigrants contribute more than 24% in detection of new cases since 1990. Treatment success rate in term of completion of treatment to date is 82%. Mortality rate has steadily declined from 14 deaths to 7 deaths per 100,000 population. Leprosy in Sabah also contributes to 30% of the yearly total caseload.
of Malaysia and has the highest notification rate of 2 per every 100,000 population as compared to other states. The average registered leprosy cases over the past 5 years are 239 cases and the prevalence rate is 0.7/10,000 population. The state has successfully achieved its goal to decrease leprosy as per the World Health Organization (WHO) goal of yearly overall prevalence rate of less than 1 case for every 10,000 population. However, the districts of Kudat, Tawau, Lahad Datu, Kota Kinabalu and Semporna are still within the prevalence rate of more than one per 10,000 population. This review highlights some interesting findings which can be incorporated into the State and Districts action plans and strategies. It is also noted that in order to translate National Plans and Strategies into effective action at the community level, health workers need relevant up-to-date knowledge of the pattern of health and disease, and of their determinants, in each district. The Sabah Health Department continues to organize and support programs related to management and control of tuberculosis and leprosy to progressively reduce the incidence of these diseases in the community by breaking the chain of transmission of *Mycobacterium tuberculosis* and *M. leprae*, respectively.—Authors’ Abstract

### Other Mycobacterial Diseases


BACKGROUND: Exposure to environmental microorganisms is associated with variations in the prevalence and severity of atopic diseases. We have previously shown that administration of a *Mycobacterium vaccae* suspension significantly reduced the severity of atopic dermatitis (AD) in children aged 5–18 years. OBJECTIVES: This study aimed to extend these observations to younger children. METHODS: Fifty-six children aged 2–6 years with moderate to severe AD were enrolled in a randomized, double-blind, placebo-controlled trial and given one intradermal injection of either killed *M. vaccae* suspension or buffer solution (placebo). Skin surface area affected and dermatitis severity score were assessed before and 1, 3 and 6 months after treatment. RESULTS: Although a 38–54% reduction in surface area affected by dermatitis was noted at all time points after *M. vaccae* administration (p = 0.005), this improvement was not significantly different from that observed in the placebo group. Meta-analysis of this and our previous cohort (97 children aged 2–18 years) showed that *M. vaccae* was associated with a significant improvement in clinical severity at all ages, whereas within the placebo group, younger but not older children showed a similar improvement. CONCLUSIONS: Despite a reduction in clinical severity associated with *M. vaccae* at all ages, no benefit could be found after administering *M. vaccae* to children with AD aged 2–6 years when compared with placebo. *M. vaccae* may offer greater benefit in children over 5 years old, whose AD appears less likely to regress spontaneously.—Authors’ Abstract


*Mycobacterium avium* subspecies paratuberculosis (*M. paratuberculosis*) is the causative agent of Johne’s disease in ruminants. *M. paratuberculosis* is a slow-growing intracellular bacterium and infections with *M. paratuberculosis* can persist in a subclinical state for several years. An early and appropriate T cell-mediated cytotoxic response (Th1-like) to *M. paratuberculosis* infection is often replaced with an antibody or Th 2-like response as infected animals move toward a progressively more clinical state. The reasons for this shift in immune response are unknown. Recent studies suggest that *in vitro* exposure of peripheral blood mononuclear cells (PBMCs) from
Johne’s disease positive cows to *M. paratuberculosis* for 18–24 hr results in suppressed expression of numerous immune cell genes. This effect appears at odds with the notion that immune cells from infected cows would respond to *M. paratuberculosis*-specific antigens in a vigorous and positive manner. In this report, we detail experiments designed to test the hypothesis that many positive changes in PBMC gene expression induced by *M. paratuberculosis in vitro* are transient, being rapidly suppressed by as yet unknown mechanisms. Our results demonstrate that, indeed, in vitro stimulation with *M. paratuberculosis* induces rapid changes in infected cow PBMC gene expression (within 2–4 hr of exposure) and that many of these changes are no longer evident by 8–16 hr of exposure to *M. paratuberculosis*. Although precise mechanisms responsible for rapid *M. paratuberculosis*-mediated activation of PBMC gene expression and the loss thereof remain to be determined, our novel results suggest that PBMCs from Johne’s disease positive cows are indeed capable of vigorously responding to *M. paratuberculosis* and that, for many genes, this response is tempered within 8 hr of exposure.—Authors’ Abstract


*Mycobacterium ulcerans* causing Buruli ulcer is an environmental mycobacteria responsible for an infectious necrotizing pan- niculitis. The epidemiology of this disabling disease is strongly linked to the aquatic ecosystem. Occurring mainly in children, it is an emergent public health threat in many humid rural tropical areas. Human contamination probably follows a direct pectaneous route from humid environment, but some insects may play a role in transmission. The clinical features develop in three phases: pre-ulcer, ulcer with unstick margins, healing leading to functional sequelae. Treatment relies on antibiotics in order to sterilize the infectious focus, together with the surgical repair of lost skin and joint deformities, as well as early physiotherapy. Despite uncertainties of *in vivo* efficacy of antibiotics, it seems logical to administer chemotherapy with both Rifampicin and Aminoglycosid or Fluroquinolon and Aminoglycosid. Surgical treatment depends on the size of the ulcer, as well as available techniques and skills on the field. Wide excision and graft are often recommended, however limited excision followed by small islet grafts may be successful.—Bulletin de la Société de Pathologie Exotique

Peritonitis is the most common complication and the leading cause of death in pediatric peritoneal dialysis (PD) patients. According to the most recent data available from the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS), approximately 25% of pediatric PD patients who die succumb to infection. There are no reported cases of Mycobacterium tuberculosis (MTB) or Mycobacterium avium-intracellulare peritonitis in the NAPRTCS registry. With an increasing incidence of MTB worldwide and the impairment of cellular immunity in chronic renal failure patients, it is not surprising that mycobacterium peritonitis can occur in PD patients. We report two pediatric PD patients with mycobacterial peritoneal infection diagnosed over an 11-year period at our institution. One patient presented with a malfunctioning Tenckhoff catheter and again 3 years later with hyponatremia and ascites. The other presented with recurrent culture-negative peritonitis. These cases illustrate the importance of more extensive evaluation of PD complications, to include evaluation for mycobacterium with special media or peritoneal biopsy, in the above clinical settings if the routine work-up is unrevealing.—Authors’ Abstract


A case of unilateral interface keratitis due to Mycobacterium fortuitum following simultaneous bilateral LASIK procedure for low myopia is reported. Excimer phototherapeutic keratectomy was performed to the stromal bed to reduce the infective load. Intensive topical therapy with topical amikacin and ciprofloxacin resulted in resolution of the keratitis.—Authors’ Abstract


The in vitro antimycobacterial activity of two ciprofloxacin analogues (2a and 2b) containing 2-phenyl-2-oxoethyl and 2-(4-fluorophenyl)-2-oxoethyl groups attached to N-4 position of piperazin ring, was evaluated against M. tuberculosis strains resistant to Isoniazid (MIC 2a and 2b = 3.13 micrograms/ml), Ethambutol (MIC 2a and 2b = 1.56 micrograms/ml), Rifampin (MIC 2a and 2b = 1.56 micrograms/ml), Kanamycin (MIC 2a and 2b = 1.56 micrograms/ml) and ciprofloxacin (MIC 2a and 2b = 5.12 micrograms/ml). Furthermore, the minimum bactericidal concentration (MBC) of 2a and 2b was determined against M. tuberculosis H37Rv (MBC2a = 6.25 and 2b = 25 micrograms/ml) and strains resistant to isoniazid (MBCs >25 micrograms/ml) and rifampin (MBC2a = 3.13 and 2b = 6.25 micrograms/ml). Also, in this study the activity of 2a and 2b was determined against a strain of M. avium (%Inhibition 2a = 89 and 2b = 95%). Expanded primary screening was conducted for 2b (having MIC <6.25 micrograms/ml) against five clinical isolates of M. avium using the MABA and BACTEC 460 systems (MIC = 2–4 micrograms/ml). The significant antimycobacterium avium activity of 2b suggested further M. avium assays and so, 2b was then retested at lower concentrations against 30 strains of M. avium including five strains resistant to clarithromycin (MIC = 2–16 micrograms/ml).—Authors’ Abstract


We report the case of a 29-year-old transsexual who developed Mycobacterium abscessus infection after receiving intramammary liquid silicone injections in the nonphysician office setting. Our patient represents 1 of 14 confirmed and 11 suspected
cases in New York City of *M. abscessus* infection after illicit cosmetic procedures. As injectable cosmetic procedures are becoming increasingly popular, dermatologists should be aware of both the common and unusual complications. Furthermore, all physicians should be alerted to the current cluster of *M. abscessus* infections after injections for cosmetic purposes by nonmedical practitioners in New York City.—Authors’ Abstract


LC-MSn analysis of mycolactone toxin from extracts of *Mycobacterium ulcerans* has shown that minor co-metabolites, including two previously unreported, differ structurally from mycolactone only in a small portion of the polyketide side-chain.—Authors’ Abstract


A rapidly growing mycobacterium was isolated five times from blood cultures from a 6-year-old female patient with relapsed pre-B-cell acute lymphocytic leukemia. All five isolates had identical nucleotide sequences for the first 500 bp of the 16S rRNA gene, indicative of a single species. High-performance liquid chromatography analysis of mycolic acids indicated that the species was similar to *Mycobacterium smegmatis*. Sequence analysis of the 16S rRNA gene (1,455 bp) for one isolate demonstrated that the species was closely related to *Mycobacterium diernhoferi*. Based on the phenotypic features and phylogenetic analysis, it was concluded that the isolates represented a novel rapidly growing Mycobacterium species. The name “Mycobacterium hackensackense” is proposed for this unique strain, 147-0552(T), which was deposited in the American Type Culture Collection as ATCC BAA-823(T).—Authors’ Abstract


See Current Literature, Microbiology, p. 222.


*Mycobacterium aurum* was cultured from the Broviac catheter of a 5-year-old child with metastatic Wilms tumor. Removal of the catheter resulted in prompt resolution of the fever and sterilization of the blood culture. This rapidly growing mycobacterium, previously believed to be a commensal, can cause disease in the immunocompromised host.—Authors’ Abstract


*Mycobacterium fortuitum* is a rapidly growing, nontuberculous mycobacteria that has rarely been associated with central nervous system impairment. We describe the case of a patient who developed multiple cerebral abscesses revealing *Mycobacterium fortuitum* infection. Brain biopsy specimens showed suppurative, noncaseating, granulomatous inflammation consisting of epithelioid histiocytes and multinucleated giant cells. All clinical signs and CT scan cerebral...
lesions disappeared after institution of appropriate antimycobacterial therapy.—Authors’ Abstract


The effectiveness of rifampicin (RIF), amikacin (AMK) and their combination were estimated in the treatment of mice experimentally infected by Mycobacterium ulcerans and the risk of relapse after the treatment was evaluated. After 7 weeks of treatment with RIF or with the combination of AMK/RIF and 8 weeks with AMK alone, no viable bacilli were found in the infected tissues and these remained uninfected during the following 6 months. Among the mice treated with AMK alone, three mice relapsed, but the minimal inhibitory concentration of AMK for these isolates remained unchanged. With RIF alone, two mice relapsed and the minimal inhibitory concentration of these isolated strains was higher. However, with all the mice treated with both RIF and AMK, no relapse was observed.—Authors’ Abstract


Mycobacterium haemophilum, a strongly acid- and alcohol-fast bacillus belonging to the group of non-tuberculous mycobacteria was first described in 1978 as the cause of cutaneous ulcerating lesions in a woman with Hodgkin’s disease. Infection due to M. haemophilum is rare but increasing in prevalence in immunosuppressed subjects, particularly in patients with acquired immunodeficiency syndrome (AIDS) patients. The skin is the most common site of infection with erythematous or violaceous papules and/or nodules that are usually painless at first, but some elements develop into abscesses or ulcers that can become very painful. The incidence of M. haemophilum is unknown, but cases of infection have been reported in Australia, Canada, the United States, France, Israel, the United Kingdom and Taiwan; to date no cases have been reported in Italy, thus the case reported here is apparently the first one observed in our country.—Authors’ Abstract


Mycobacterium ulcerans infection is the third most important mycobacterial infection in the world. It has been described in many different countries including French Guiana. The diagnosis of M. ulcerans infection by culture is often difficult because culture is hard to perform in endemic areas and their sensitivity is not reliable. As a result the diagnosis of this infection is often delayed. However, molecular methods are now available to diagnose rapidly infections by M. ulcerans and distinguish it from other mycobacteria. We report three cases of skin infection due to M. ulcerans observed in French Guiana. Diagnosis was initially made by polymerase chain reaction and was confirmed later by culture (in two patients) and inoculation to mice (in one patient). A faster diagnosis of M. ulcerans infection should lead to a better prognosis of this infection.—Bulletin de la Société de Pathologie Exotique


Pulmonary disease caused by nontuberculous mycobacteria (NTM) is a relatively rare occurrence in immunocompetent children. Two cases of endobronchial NTM infection in immunocompetent children are described. In addition, 41 other children...
With NTM pulmonary disease reported in the English literature between 1930 and 2003 are reviewed. Clinical manifestations are either purely respiratory or respiratory with more widespread systemic symptoms. Compared with children with only respiratory complaints, children with constitutional symptoms from NTM pulmonary disease had symptoms for a shorter period before presentation (10 vs 28 days), 2) had more radiographic evidence of pulmonary disease, and 3) were treated longer with antimycobacterial agents (11.5 months vs 6 months). The most common causative organism was \textit{Mycobacterium avium} complex. Pediatricians should be increasingly aware of NTM in the differential diagnosis of persistent pulmonary disease in previously healthy children.—Authors’ Abstract


With the increasing lifespan of persons with cystic fibrosis (CF), the emergence of a variety of previously seldom seen pathogens, including the nontuberculous mycobacteria (NTM), has been seen. Determining the impact of these indolent organisms on the natural history of cystic fibrosis lung disease has been difficult. We initiated a two-stage study in 1993 to assess the prevalence and clinical impact of these organisms among persons with CF in US CF Centers. These organisms were frequently recovered from older patients with relatively mild disease. While over the short, 15-month course of follow-up no significant differences in the rate of decline of lung function attributable to NTM were seen, concerning changes and progression of high-resolution computed tomography findings were seen in patients from whom these organisms were repeatedly recovered.—Authors’ Abstract


See Current Literature, Experimental Infections, p. 238.


Despite the increasing prevalence of cervicofacial lymphadenitis due to atypical mycobacteria (AMB) in children, the true nature of AMB infection in clinical practice is poorly understood. The purpose of our study was to define the most common signs and symptoms, and to establish a workable scheme of diagnosis and treatment. Patients fulfilling the criteria of AMB infection (i.e., clinical signs, positive cultures or polymerase chain reaction, histologic features) were included in the study. All children underwent a standard surgical procedure, depending on pretreatment and the course of the disease. Sixteen infants presented with characteristic unilateral lymphadenopathy predominantly involving the submandibular area (13/16). Eight children had been initially treated at various institutions by fine-needle puncture or incision, and 7 of the 16 patients had received antituberculous multidrug treatment for a varying length of time. Complete excision of the affected lymph nodes was the definitive treatment in all patients. Three children had transient marginal mandibular nerve paralysis that resolved within a few months in all cases. Recognition of the characteristic features of AMB adenitis may permit early diagnosis and appropriate surgical treatment.—Authors’ Abstract


OBJECTIVE: To discuss the clinical aspects and management of nontuberculous mycobacteriosis of the temporal bone.
STUDY DESIGN: Case report and review of the literature. SETTING: University hospital, tertiary referral center. PATIENT, INTERVENTION, AND RESULTS: The authors describe an uncommon case of non-tuberculous mycobacteriosis of the temporal bone in an immunosuppressed 62-year-old woman with facial nerve paralysis caused by disease complication. The case was cured with radical tympanomastoidectomy and prolonged multiple antibiotic therapy. CONCLUSIONS: Nontuberculous mycobacteriosis should be suspected in immunosuppressed patients with intractable middle ear granulations. Cultural and histologic examinations are the mainstay for diagnosis. Long-standing multiantibiotic therapy together with aggressive surgery should be considered as appropriate management.—Authors’ Abstract


Buruli ulcer is an extensive ulceration usually on the extremities. The ulcer can spread to subcutaneous fat, muscle and even bone causing osteomyelitis and death. It is the third most common mycobacterial disease in humans after tuberculosis and leprosy. The bacterium grows in still standing water and infects children through small ulcerations in their skin. Mycobacterium ulcerans may also be transmitted by the bite of aquatic bugs (Naucordiae), which harbor the bacterium in their salivary glands. The disease affects poor people in rural, tropical areas where deforestation has led to flooding rivers, stagnant bodies of water and marsh. Benin, Cote d’Ivoire and Ghana in West Africa are seriously hit. Skin transplantation is the treatment of choice. Treatment with antibiotics has been disappointing.—Author’s Abstract


The genus Mycobacterium consists of >50 species that have been associated with human disease. Mycobacterium are categorised into M. tuberculosis and NTM that are also subdivided into rapid growers and non-rapid growers. Five major clinical syndromes have been described that are attributable to mycobacterium. These include: pulmonary disease; lymphadenitis; skin, soft tissue, and skeletal infections; catheter-related blood-stream infections in immunocompromised hosts; and disseminated disease in persons with AIDS. There is very limited documentation of person-to-person transmission of NTM. Nosocomial infections and outbreaks caused by inadequate disinfection/sterilization of medical devices or environmental contamination of medications or medical devices are well described. Staining for AFB, culture, histopathologic, or genetic amplification technologies are used to detect and identify mycobacterium. Pulsed-field gel electrophoresis is the method of choice to determine strain relatedness. At present, susceptibility testing for non-tuberculous mycobacteria is not fully standardized and has not been correlated with clinical outcomes.—Authors’ Abstract


A 65-year-old woman, treated with prednisolone (5 mg daily) for rheumatoid arthritis, visited our hospital because of right chest pain. Chest CT showed small nodular shadows in the right lung accompanied with right pleural effusion. A pulmonary Mycobacterium gordonae infection was diagnosed, since M. gordonae was identified twice from her sputum. She was treated with rifampicin, ethambutol and streptomycin for two months, and then streptomycin was replaced with clarithromycin. Three months after the initial treatment, M. gordonae was eradicated from her sputum. Pleural puncture revealed bloody, exudative, lymphocytotic pleural effusion, but no malignant cells were identified. Although pathological diagnosis by thoracoscopic pleural biopsy could not be performed, it is likely that the pleural effusion was associated with the pulmonary M. gordonae infection in the present case.—Authors’ Abstract

Crohn’s disease is a non-specific chronic transmural inflammatory disease. The disease was associated with a frameshift mutation in the NOD2 gene. Nevertheless, other researchers associated the presence of M. paratuberculosis within the intestinal tissues of patients with the disease. An adapted “in situ hybridization” technique was used to detect IS900 M. paratuberculosis DNA in paraffin embedded tissue from Crohns tissue disease samples. We were able to identify M. paratuberculosis DNA in around 69% of the paraffine embedded intestinal samples of Crohn’s disease patients analysed. The presence of M. paratuberculosis DNA in the intestinal samples analysed does not necessarily mean that M. paratuberculosis is responsible for Crohn’s disease. Our results support the hypothesis that infection may be caused by cell wall defective M. paratuberculosis since no bacteria were detected by Ziehl Neelsen stain.—Authors’ Abstract


We prospectively studied 298 patients with cystic fibrosis (mean age 11.3 years; range 2 months to 32 years; sex ratio, 0.47) for nontuberculous mycobacteria in respiratory samples from January 1, 1996, to December 31, 1999. Mycobacterium abscessus was by far the most prevalent nontuberculous mycobacterium: 15 patients (6 male, 9 female; mean age 11.9 years; range 2.5–22 years) had at least one positive sample for this microorganism (versus 6 patients positive for M. avium complex), including 10 with >3 positive samples (versus 3 patients for M. avium complex). The M. abscessus isolates from 14 patients were typed by pulsed-field gel electrophoresis: each of the 14 patients harbored a unique strain, ruling out a common environmental reservoir or person-to-person transmission. Water samples collected in the cystic fibrosis center were negative for M. abscessus. This major mycobacterial pathogen in children and teenagers with cystic fibrosis does not appear to be acquired nosocomially.—Emerging Infectious Diseases


BACKGROUND: Mycobacterium avium causes disseminated infection in immuno-compromised patients and triggers a process resembling Crohn’s disease in goats. Colony morphotypes predict pathogenicity. Smooth-transparent (SmT) morphotypes are more virulent and induce less interleukin (IL)-1beta and IL-18 production than avirulent smooth-domed (SmD) morhotypes. Caspases are essential for IL-1beta and IL-18 production. METHODS: Caspase activation was examined in human monocytes after M. avium infection. RESULTS: Fresh monocytes constitutively expressed caspase-1 mRNA and pro-caspase-1. The M. avium infection increased monocyte caspase-1 mRNA expression. Furthermore, SmD-infected monocytes expressed 2.3-fold higher levels (p <0.05, N = 3) of activated caspases than SmT-infected monocytes. Caspase-1 inhibition significantly reduced IL-1beta production by SmT- and SmD-infected monocytes (p <0.05, N = 4). Caspase-3 inhibition inhibited IL-1beta production 43.5% ± 8.0% (p <0.02, N = 4) by SmD-infected but not SmT-infected monocytes. CONCLUSIONS: Decreased mature IL-1beta release by SmT-infected monocytes may reflect selective induction of caspase-1 activity but not caspase-3. Differential caspase expression in monocytes after infection may contribute to M. avium pathogenicity in humans.—Authors’ Abstract

Sugihara, E., Hirota, N., Niizeki, T., Tanaka, R., Nagafuchi, M., Koyanagi,

To evaluate the usefulness of bronchial lavage for the diagnosis of pulmonary disease due to Mycobacterium avium-intracellulare complex (MAC) infection, we examined the clinical records and bacteriologic findings of patients admitted to our hospital between 1999 and 2002 who fulfilled the 1997 American Thoracic Society (ATS) criteria for MAC pulmonary infection. Bronchoscopic examinations were performed in those patients with MAC pulmonary disease who showed negative sputum smears for mycobacteria on 3 consecutive days (N = 14) or who could not expectorate sputum (n = 2). The bronchial lavage sample was smear-positive for acid-fast bacilli in 8 of the 16 patients (50.0%), polymerase chain reaction (PCR)-positive for MAC in 10 of 15 (66.7%), and culture-positive for MAC in 15 of 16 (93.7%). The brushing sample was positive for MAC in 5 of 14 patients (35.7%), and transbronchial lung biopsy (TBLB)-positive for MAC in 2 of 5 (40.0%). MAC was isolated by culture of bronchial lavage samples in a higher percentage of patients than that in whom MAC was isolated by sputum culture, and we could make an early diagnosis of MAC pulmonary disease based on the smear and PCR results for bronchial lavage samples. Bronchial lavage is useful to screen sputum smear-negative patients suspected of having MAC pulmonary disease.—Authors’ Abstract


Objectives: Assess treatment effects by following up patients treated for Buruli ulcer in two hospitals with different treatment aspects, including widely differing surgical practices. Patients/methods: Treated patients were retrospectively identified from hospital records. Between 1994 and July 2000, 136 patients had been admitted for Buruli ulcer in both hospitals, and lived in areas covered in the research period. 78 (57%) Patients were included in the study. Treatment and status of the patient were analyzed. Results: 27 (35%) Patients were not healed. Of the 33 patients treated in hospital A, six (18%) were not healed at follow-up, whereas of the 45 patients treated in hospital B, 21 (47%) were not healed. The length of stay in hospital A was significantly longer (p = 0.002), and more operations on average were done per patient (p = 0.002). In a univariate analysis, treatment in hospital A; the use of rifampicin (p = 0.013); and BCG vaccination status (p = 0.04) were all significantly associated with ulcer healing. Using a logistic regression model for multivariate analysis, only treatment as given in hospital A, with standard practice of wide surgical excision, appeared to predict ulcer healing independently (p = 0.02). Conclusions: This study shows large differences in treatment outcome between the two hospitals; the results support the hypothesis that extent of surgical treatment influences the chance of healing of Buruli ulcer.—Tropical Disease Bulletin


Non tuberculous Mycobacterial (NTM) infections mainly affect immunocompromised patients, appearing as disseminated or pulmonary disease. In immunocompetent children the most common form of infection with NTM is cervical adenitis. Ear infection seems to be a rare disease. We present a case of otomastoiditis caused by Mycobacterium avium in a 15 months old child, immunologically normal. Patient was referred for persistent right otitis unresponsive to routine medical therapy. TC scan of the ear and temporal bones revealed: soft tissue in external auditory canal, Eustachian canal, and middle ear overlying ossicles with erosion of tegmen tympani. Tuberculin skin test was positive
with 5 units PPD and culture yielded \textit{M. avium}. The patient undergo timpanomastoidectomy and medical therapy with antituberculous drugs and steroids, subsequently he was given Clarithromycin and Rifabutin. \textit{M. avium} is an ubiquitous low grade pathogen found in soil, water, dust and food. There is no evidence of direct transmission. Only a few cases of otomastoiditis due to \textit{M. avium} have previously been reported. The case presented underlines the importance of microbiological investigations. When a NTM infection is suspected surgeons and infectious diseases specialists should cooperate to find an optimal treatment regimen of this unusual disease.—Authors’ Abstract


\textit{Mycobacterium abscessus} is a rare cause of skin and soft tissue infections that often results from inoculation with contaminated foreign material. A 41-year-old woman is described regarding an outbreak of \textit{M. abscessus} following soft tissue augmentation. Clinical features and treatment options are reviewed.—Author’s Abstract


Nontuberculous mycobacterial infections are becoming more common. Recently, \textit{Mycobacterium fortuitum} and other rapidly growing mycobacteria have been found to cause severe skin and soft-tissue infections in association with nail salon whirlpool footbaths. We recently investigated a large outbreak of \textit{M. fortuitum} furunculosis among women who received pedicures at a single nail salon. To better define the clinical course of such infections, we collected clinical details from physicians who were treating outbreak patients. We constructed multivariable linear models to evaluate the effect of antibiotic treatment on disease duration. Sixty-one patients were included in the investigation. The mean disease duration was 170 days (range, 41–336 days). Forty-eight persons received antibiotic therapy for a median period of 4 months (range, 1–6 months), and 13 persons were untreated. Isolates were most susceptible to ciprofloxacin and minocycline. Early administration of therapy was associated with shorter duration of disease only in persons with multiple boils (p <.01). One untreated, healthy patient had lymphatic disease dissemination.—Authors’ Abstract


See Current Literature, Microbiology, p. 219.


The secreted 24 kDa lipoprotein (LppX) is an antigen that is specific for \textit{Mycobacterium tuberculosis} complex and \textit{M. leprae}. The present study was carried out to identify the promiscuous T helper 1 (Th1)-cell epitopes of the \textit{M. tuberculosis} LppX (MT24, Rv2945c) antigen by using 15 overlapping synthetic peptides (25 mers overlapping by 10 residues) covering the sequence of the complete protein. The analysis of Rv2945c sequence for binding to 51 alleles of nine serologically defined HLA-DR molecules,
by using a virtual matrix-based prediction program (propred), showed that eight of the 15 peptides of Rv2945c were predicted to bind promiscuously to ≥10 alleles from more than or equal to three serologically defined HLA-DR molecules. The Th1-cell reactivity of all the peptides was assessed in antigen-induced proliferation and interferon-gamma (IFN-gamma)-secretion assays with peripheral blood mononuclear cells (PBMCs) from 37 bacille Calmette-Guerin (BCG)-vaccinated healthy subjects. The results showed that 17 of the 37 donors, which represented an HLA-DR-heterogeneous group, responded to one or more peptides of Rv2945c in the Th1-cell assays. Although each peptide stimulated PBMCs from one or more donors in the above assays, the best positive responses (12/17 (71%) responders) were observed with the peptide p14 (aa 196–220). This suggested a highly promiscuous presentation of p14 to Th1 cells. In addition, the sequence of p14 is completely identical among the LppX of Mycobacterium tuberculosis, Mycobacterium bovis and Mycobacterium leprae, which further supports the usefulness of Rv2945c and p14 in the subunit vaccine design against both tuberculosis and leprosy.—Authors’ Abstract


See Current Literature, Clinical Sciences, p. 192.


We report 2 isolates of Mycobacterium fortuitum from patients with pulmonary tuberculosis lesions hybridizing to IS6110 probe in restriction fragment length polymorphism (RFLP) typing. Results of polymerase chain reaction-hybridization formats using the non-specific region of IS6110 for the molecular detection of mycobacteria in clinical material should be interpreted with caution.—Authors’ Abstract


A recombinant DNA strategy was applied to analyze and screen the shotgun expression library from a clinically confirmed local virulent isolate of Mycobacterium tuberculosis with sera from tuberculosis patients, which led to expression and purification of highly immunoreactive and specific mycobacterial antigens expressed during the course of active disease which could be of diagnostic significance. An enzyme-linked immunoassay for diagnosis of tuberculosis was devised by using a shotgun immunoprobe expression library in the lambda gt11 vector. DNA from a virulent M. tuberculosis patient isolate (TBW-33) confirmed with the BACTEC 460 system was sheared and expressed to generate shotgun polypeptides. beta-Galactosidase fusion proteins capable of demarcating active tuberculosis infections from Mycobacterium bovis BCG-vaccinated healthy subjects or people harboring environmental mycobacteria were selected by comparative immunoreactivity studies. Promising mycobacterial DNA casettes were subcloned and expressed into the glutathione S-transferase (GST) fusion vector pGEX-5X-1 with a strong tac promoter and were expressed in Escherichia coli BL21. These fusion proteins were severed at a built-in factor Xa recognition site to separate the GST tags and were utilized in an indirect enzyme-linked immunoassay for serodiagnosis of patients with active tuberculosis. The system offered a clear demarcation between BCG-vaccinated healthy subjects and patients with active tuberculosis and proved to be effective in detecting
pulmonary as well as extrapulmonary tuberculosis, with an overall sensitivity of 84.33% and an overall specificity of 93.62%.

—Authors’ Abstract


Four putative promoters of the temperate mycobacteriophage L1 were cloned by detecting the beta-galactosidase reporter expression in E. coli transformants that carried L1 specific operon-fusion library. All of the four L1 promoters were also found to express differentially in the homologous environment of mycobacteria. Of the four promoters, two were suggested to be the putative early promoters of L1 since they express within 0 to 10 min of the initiation of the lytic growth of L1. One of the putative early promoters showed a relatively better and almost identical activity in both E. coli and M. smegmatis. By a sequence analysis, we suggest that the L1 insert that contained the stronger early promoter possibly carries two convergent E. coli sigma70-like L1 promoters, which are separated from each other by about 300 nucleotides. One of them is the early promoter of L1 as it showed a 100% similarity with the early Pleft promoter of the homimmune phage L5. The second promoter, designated P4, was suggested for its appreciable level of reporter activity in the absence of the -10 element of the Pleft equivalent of L1. By analyzing most of the best characterized mycobacteriophages-specific promoters, including the L1 promoter P4, we suggest that both the -10 and -35 hexamers of the mycobacteriophage promoters are highly conserved and almost similar to the consensus -10 and -35 hexamers of the E. coli sigma70 promoters.

—Authors’ Abstract


A new strategy known as multiplex PCR amplimer conformation was developed for detection of mutation in the gyrA gene of 138 clinical isolates of Mycobacterium tuberculosis. The method generated a single-stranded and heteroduplex DNA banding pattern of multiplex PCR amplimers of the region of interest that was extremely sensitive to specific mutations, thus enabling much more sensitive and reliable mutation analysis compared to the standard single-stranded conformation polymorphism technique. The genetic profiles of the gyrA gene of the 138 isolates as detected by MPAC were confirmed by nucleotide sequencing and were found to correlate strongly with the in vitro susceptibilities of the mutant strains to six fluoroquinolones (ofloxacin, levofloxacin, sparfloxacin, moxifloxacin, gatifloxacin, and sitafloxacin). All 32 isolates that contained gyrA mutations exhibited cross-resistance to the six fluoroquinolones (ofloxacin MIC for 90% of strains >16 mg/liter), although moxifloxacin, gatifloxacin, and sitafloxacin (MIC for 90% of strains ≤4 mg/liter) were apparently more active than ofloxacin, levofloxacin, and sparfloxacin (MIC for 90% of strains >/>=2” BORDER=’” >16 mg/liter). All gyrA mutations were clustered in codons 90, 91, and 94, and aspartic acid 94 was most frequently mutated. Twenty-three isolates without gyrA mutations were also found to exhibit reduced susceptibility to ofloxacin (MIC for 90% of strains = 4 mg/liter), but largely remained susceptible to other drugs (MIC for 90% of strains ≤1 mg/liter). Another 83 isolates without mutations were fully susceptible to all six fluoroquinolones (ofloxacin MIC for 90% of strains = 1 mg/liter). In conclusion, high-level phenotypic resistance to fluoroquinolones among M. tuberculosis clinical isolates, which appears to be predominantly due to gyrA mutations, may be readily detected by genotyping techniques such as multiplex PCR amplimer conformation.

—Authors’ Abstract


The region of conserved synteny on mouse chromosome 11/human 17q11-q21 is known to carry a susceptibility gene(s) for intra-macrophage pathogens. The region is rich in candidates including NOS2A, CCL2/MCP-1, CCL3/MIP-1alpha, CCL4/MIP-1beta,CCL5/RANTES, CCR7, STAT3 and STAT5A/5B. To examine the region in man, we studied 92 multicase tuberculosis (627 individuals) and 72 multicase leprosy (372 individuals) families from Brazil. Multipoint nonparametric analysis (ALLEGRO) using 16 microsatellites shows two peaks of linkage for leprosy at D17S250 (Z(lr) score 2.34; p = 0.01) and D17S1795 (Z(lr) 2.67; p = 0.004) and a single peak for tuberculosis at D17S250 (Z(lr) 2.04; p = 0.02). Combined analysis shows significant linkage (peak Z(lr) 3.38) at D17S250, equivalent to an allele sharing LOD score 2.48 (p = 0.0004). To determine whether one or multiple genes contribute, 49 informative single nucleotide polymorphisms were typed in candidate genes. Family-based allelic association testing that was robust to family clustering demonstrated significant associations with tuberculosis susceptibility at four loci separated by intervals (NOS2A-8.4 Mb-CCL18-32.3 kb-CCL4-6.04 Mb-STAT5A/5B) up to several Mb. Stepwise conditional logistic regression analysis using a case/pseudo-control data set showed that the four genes contributed separate main effects, consistent with a cluster of susceptibility genes across 17q11.2.—Authors’ Abstract


The species identification within Mycobacterium terrae complex has been known to be very difficult. In this study, the genomic diversity of M. terrae complex with eighteen clinical isolates, which were initially identified as M. terrae complex by phenotypic method, was investigated, including that of three type strains (M. terrae, M. nonchromogenicum, and M. triviale). 16S rRNA and 65-kDa heat shock protein (hsp 65) gene sequences of mycobacteria were determined and aligned with eleven other references for the comparison using similarity search against the GenBank and Ribosomal Database Project II (RDP) databases. 16S rRNA and hsp 65 genes of M. terrae complex showed genomic heterogeneity. Amongst the eighteen clinical isolates, nine were identified as M. nonchromogenicum, eight as M. terrae, one as M. mucogenicum with the molecular characteristic of rapid growth. M. nonchromogenicum could be subdivided into three subgroups.


A multilayered feed-forward ANN architecture trained using the error-back-propagation (EBP) algorithm has been developed for predicting whether a given nucleotide sequence is a mycobacterial promoter sequence. Owing to the high prediction capability (congruent with 97%) of the developed network model, it has been further used in conjunction with the caliper randomization (CR) approach for determining the structurally/functionally important regions in the promoter sequences. The results obtained thereby indicate that: (i) upstream region of -35 box, (ii) -35 region, (iii) spacer region and, (iv) -10 box, are important for mycobacterial promoters. The CR approach also suggests that the -38 to -29 region plays a significant role in determining whether a given sequence is a mycobacterial promoter. In essence, the present study establishes ANNs as a tool for predicting mycobacterial promoter sequences and determining structurally/functionally important sub-regions therein.—Authors’ Abstract
while *M. terrae* could be subdivided into two subgroups using a 5 bp criterion (>1% difference). Seven isolates in two subgroups of *M. nonchromogenicum* were *Mycobacterium* sp. strain MCRO 6, which was closely related to *M. nonchromogenicum*. The hsp 65 gene could not differentiate one *M. nonchromogenicum* from *M. avium* or one *M. terrae* from *M. intracellulare*. The nucleotide sequence analysis of 16S rRNA and hsp 65 genes was shown to be useful in identifying the *M. terrae* complex, but hsp 65 was less discriminating than 16S rRNA.—Authors’ Abstract


OBJECTIVE: To investigate association between the natural-resistance-associated macrophage protein 1 (NRAMP1) gene polymorphisms and susceptibility to pulmonary tuberculosis (TB) in Chinese Han population. METHODS: Hospital-based case-control study design was adopted. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique were used to type three NRAMP1 polymorphisms (INT4, D543N and 3′UTR). Information on related factors of tuberculosis was collected using a pre-tested standard questionnaire. Univariate and multivariate unconditional logistic analyses were conducted using SPSS for window software package. Totally, 110 cases of TB were selected during April 2001 to June 2002, with an average age of (27.7 ± 12.7) years. Also, 180 cases of healthy control were selected, aged (27.3 ± 9.2) years in average. Locus of NRAMP1 polymorphism was analysed with univariate method. RESULTS: Univariate analysis demonstrated that the D543N G/A and 3′UTR TGTG+/del genotype occurred more frequently in the cases than in the controls, with crude odds ratios (OR) (95% CI) of 2.22 (1.03–4.78) and 1.93 (1.14–3.26), respectively. No significant association was observed between TB and INT4 polymorphisms. In multivariate analysis, associations of TB and D543N G/A and 3′UTR TGTG+/del genotypes remained, adjusted for exposure history and bacille Calmette-Guerin immunization. Adjusted OR (95% CI) was 3.04 (1.12–8.27) and 2.36 (1.20–4.64), respectively. Still, no significant association between INT4 polymorphisms and TB was found. CONCLUSION: Polymorphisms of D543N and 3′UTR locus in NRAMP1 gene might affect their susceptibility to TB in Chinese Han population.—Authors’ Abstract


SUMMARY: AmpliBASE MT trade mark is an online databank of high-resolution DNA fingerprints representing fluorescent amplified fragment length polymorphism (FAFLP) profiles or amplitypes developed for the *Mycobacterium tuberculosis* complex strains from 48 different countries. AmpliBASE MT trade mark is based on a relational database management system that is hyperlinked to visualize genotyping results in the form of DNA fingerprint images for individual strains. A flexible search system based on systematic comparisons of fragment sizes in base pairs allows inter-laboratory comparison of FAFLP profiles. Besides this, the database also displays previously published data on IS6110 profiles, spoligotypes, MIRU-VNTRs and large sequence polymorphisms along with the FAFLP records that will give the overall comparisons. Being the first of its kind, AmpliBASE MT trade mark is expected to be a very helpful tool in strengthening the concept of ‘geographic genomics’ and will be very helpful to molecular epidemiologists and those interested in diagnostic development for tuberculosis.—Authors’ Abstract

Marmiesse, M., Brodin, P., Buchrieser, C, Gutierrez, C., Simoes, N., Vincent, V.,

To better understand the biology and the virulence determinants of the two major mycobacterial human pathogens Mycobacterium tuberculosis and Mycobacterium leprae, their genome sequences have been determined recently. In silico comparisons revealed that among the 1439 genes common to both M. tuberculosis and M. leprae, 219 genes code for proteins that show no similarity with proteins from other organisms. Therefore, the latter ‘core’ genes could be specific for mycobacteria or even for the intracellular mycobacterial pathogens. To obtain more information as to whether these genes really are mycobacteria-specific, they were included in a focused macroarray, which also contained genes from previously defined regions of difference (RD) known to be absent from Mycobacterium bovis BCG relative to M. tuberculosis. Hybridization of DNA from 40 strains of the M. tuberculosis complex and in silico comparison of these genes with the near-complete genome sequences from Mycobacterium avium, Mycobacterium marinum and Mycobacterium smegmatis were undertaken to answer this question. The results showed that among the 219 conserved genes, very few were not present in all the strains tested. Some of these missing genes code for proteins of the ESAT-6 family, a group of highly immunogenic small proteins whose presence and number is variable among the genomically highly conserved members of the M. tuberculosis complex. Indeed, the results suggest that, with few exceptions, the ‘core’ genes conserved among M. tuberculosis H37Rv and M. leprae are also highly conserved among other mycobacterial strains, which makes them interesting potential targets for developing new specific anti-mycobacterial drugs. In contrast, the genes from RD regions showed great variability among certain members of the M. tuberculosis complex, and some new specific deletions in Mycobacterium canettii, Mycobacterium microti and seal isolates were identified and further characterized during this study. Together with the distribution of a particular 6 or 7 bp micro-deletion in the gene encoding the polyketide synthase pks15/1, these results confirm and further extend the revised phylogenetic model for the M. tuberculosis complex recently presented.—Authors’ Abstract


See Current Literature, Microbiology, Leprosy, p. 224.


Genome-wide scans were conducted for tuberculosis and leprosy per se in Brazil. At stage 1, 405 markers (10 cM map) were typed in 16 (178 individuals) tuberculosis and 21 (173 individuals) leprosy families. Nonparametric multipoint analysis detected 8 and 9 chromosomal regions respectively with provisional evidence (p <0.05) for linkage. At stage 2, 58 markers from positive regions were typed in a second set of 22 (176 individuals) tuberculosis families, with 22 additional markers typed in all families; 42 positive markers in 50 (192 individuals) new leprosy families, and 30 additional markers in all families. Three regions (10q26.13, 11q12.3, 20p12.1) retained suggestive evidence (peak LOD scores 1.31, 1.85, 1.78; p = 0.007, 0.0018, 0.0021) for linkage to tuberculosis, 3 regions (6p21.32, 17q22, 20p12) to leprosy (HLA-DQA, 3.23, p = 5.8 × 10(–5); D17S1868, 2.38, p = 0.0005; D20S889, 1.51, p = 0.004). The peak at D20S889 for leprosy is 3.5 Mb distal to that reported at D20S115 for leprosy in India. (151 words).—Authors’ Abstract
Mostowy, S., Cousins, D., and Behr, M. A.

Despite their remarkable genetic homology, members of the Mycobacterium tuberculosis complex express very different phenotypes, most notably in their spectra of clinical presentation. For example, M. tuberculosis is regarded as pathogenic to humans, whereas members having deleted RD1, such as Mycobacterium microti and Mycobacterium bovis BCG, are not. The dassie bacillus, an infrequent variant of the M. tuberculosis complex characterized as being most similar to M. microti, is the causative agent of tuberculosis (TB) in the dassie (Procavia capensis). Intriguingly, the dassie bacillus is not pathogenic to rabbits or guinea pigs and has never been documented to infect humans. Although it was identified more than a half-century ago, the reasons behind its attenuation are unknown. Because large sequence polymorphisms have presented themselves as the most obvious genomic distinction among members of the M. tuberculosis complex, the DNA content of the dassie bacillus was interrogated by Affymetrix GeneChip to identify regions that are absent from it but present in M. tuberculosis H37Rv. Comparison has led to the identification of nine regions of difference (RD), five of which are shared with M. microti (RDs 3, 7, 8, 9, and 10). Although the dassie bacillus does not share the other documented deletions in M. microti (RD1(mic), RD5(mic), MID1, MID2, and MID3), it has endured unique deletions in the regions of RD1, RD5, N-RD25, and Rv3081–Rv3082c (virS). RD1(das), affecting only Rv3874–Rv3877, is the smallest natural deletion of the RD1 region uncovered and points to genes within this region that are likely implicated in virulence. Newfound deletions from the dassie bacillus are discussed in relation to their evolutionary and biological significance.—Authors’ Abstract


Previous studies have described IS6110-mediated polymorphism as an important driving force in Mycobacterium tuberculosis genome evolution and have provided indirect evidence for IS6110-driven deletion events. This study provides the first description of an IS6110-mediated deletion event in truly isogenic strains. We also provide further support for the hypothesis that the region from Rv1754 to Rv1765 is a hot spot for IS6110 insertion and deletion events.—Authors’ Abstract


SigH, an alternative sigma factor of Mycobacterium tuberculosis, is a central regulator of the response to oxidative and heat stress. Exposure to these stresses results in increased expression of sigH itself, and of genes encoding additional regulators and effectors of the bacterial response to these stresses. In this work we show that RshA, a protein encoded by a gene in the sigH operon, is an anti-sigma factor of SigH. We demonstrate that RshA binds to SigH in vitro and in vivo. This protein-protein interaction, as well as the ability of RshA to inhibit SigH-dependent transcription, is redox-dependent, with RshA functioning as a negative regulator of SigH activity only under reducing conditions. The interaction of SigH and RshA is also disrupted in vitro by elevated temperature. RshA, a protein of 101 amino acids, contains five conserved cysteine residues of which two appear to be essential for RshA to inhibit SigH activity, suggesting that these cysteines may be important for the redox state dependence of RshA function. Our results indicate that RshA is a sensor that responds to oxidative stress, and also to heat stress, resulting in activation of SigH and expression of the SigH-dependent genes that allow M. tuberculosis to adapt to these stresses.—Authors’ Abstract


We have used representational difference analysis to identify a novel Mycobacterium avium subsp. paratuberculosis-specific ABC transporter operon (mpt), which comprises six open reading frames designated mpta to -F and is immediately preceded by two putative Fur boxes. Functional genomics revealed that the mpt operon is flanked on one end by a fep cluster encoding proteins involved in the uptake of Fe(3+) and on the other end by a sid cluster encoding non-ribosome-dependent heterocyclic siderophore synthases. Together these genes form a 38-kb M. avium subsp. paratuberculosis-specific locus flanked by an insertion sequence similar to IS1110. Expression studies using Western blot analyses showed that MptC is present in the envelope fraction of M. avium subsp. paratuberculosis. The MptD protein was shown to be surface exposed, using a specific phage (fMptD) isolated from a phage-peptide library, by differential screening of Mycobacterium smegmatis transformants. The phage fMptD-derived peptide could be used in a peptide-mediated capture PCR with milk from infected dairy herds, thereby showing surface-exposed expression of the MptD protein in the host. Together, these data suggest that the 38-kb locus constitutes an M. avium subsp. paratuberculosis pathogenicity island.—Authors’ Abstract


We have here applied high-throughput amplified fragment length polymorphism (htAFLP) analysis to strains belonging to the five classical species of the Mycobacterium tuberculosis complex. Using 20 strains, three enzyme combinations and eight selective amplification primer pairs, 24 AFLP reactions were performed per strain. Overall, this resulted in 480 DNA fingerprints and more than 1200 htAFLP-amplified PCR fragments were visualised per strain. The cumulative dendrogram correctly clustered strains from the various species, albeit
within a distance of 6.5% for most of them. The single isolate of *Mycobacterium canetti* presented separately at 19% distance. All over, 169 fragments (14%) appeared to be polymorphic. Sixty-eight were specific for *M. canetti* and forty-five for *Mycobacterium bovis*. For the 10 different *M. tuberculosis* strains included in the present analysis, 56 polymorphic markers were identified. Upon sequencing 20 of these marker regions and comparisons with the H37Rv genome sequence, 25% appeared to share homology to members of the antigenically variable PE/PPE surface protein encoding gene family confirming previous findings on the genetic heterogeneity within these genes. In addition, homologues for phage genes and insertion element-encoded genes were detected. Forty-five percent of the sequences derived from ORFs with a currently unknown function, which was corroborated by genome sequence comparison for the clinical *M. tuberculosis* CD 1551 isolate. Sequence variation in *M. tuberculosis* was assessed in more detail for a subset of these loci by newly designed PCR restriction fragment length polymorphism (RFLP) tests and direct sequencing. Fourteen novel PCR RFLP tests were developed and twelve novel single nucleotide polymorphisms (SNPs) were identified, all suited for epidemiological analysis of *M. tuberculosis*. The tests allowed for identification of the major Mycobacterium species and *M. tuberculosis* variants and clones.—Authors’ Abstract


High-density oligonucleotide microarrays allow simultaneous monitoring of the expression of a large number of cellular genes. Microarrays were used to screen the global human monocyte-derived macrophage transcriptional response to infection with the intracellular pathogen *Mycobacterium tuberculosis*. The microarray detected reproducible patterns of regulated gene expression. Analysis of the expression data showed induction of cytokines and chemokines, ribosomal proteins, and the interferon-response gene Stat1. Several changes were validated by quantitative reverse transcription polymerase chain reaction and immunoblot assays. Augmentation of the respiratory burst and preservation of the response to interferon-gamma were also demonstrated. These data supplement existing knowledge on macrophage responses to tuberculosis infection.—Authors’ Abstract
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