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Long-term Efficacy of 2 Year WHO Multiple Drug Therapy (MDT) in Multibacillary (MB) Leprosy Patients

Roland V. Cellona, Maria V. F. Balagon, Eduardo C. dela Cruz, Jasmin A. Burgos, Rodolfo M. Abalos, Gerald P. Walsh, Richard Topolski, Robert H. Gelber, and Douglas S. Walsh

ABSTRACT

Relapse rate estimates after 2 year WHO multiple drug therapy (MDT) in multibacillary (MB) leprosy vary. Between 1987 and 1994, 500 MB leprosy patients completing 2 year MDT were enrolled in a prospective relapse study. The majority of patients (N = 316) were treated and followed at the physician-staffed Cebu Skin Clinic (CSC), whereas others (N = 184) received therapy from government clinics and were followed by CSC technicians in the field. Relapse definition was an increased bacteriologic index (BI) and new skin lesions, supplemented with mouse footpad inoculations. Through 2002, follow-up was 5368 person-years, with a mean of 10.8 years per patient. The absolute relapse rate was 3% (15/498; 0.28/100 person-years), with a cumulative risk estimate of 3.9% at 15 yrs. For a subset of 217 patients followed for ≥12 yrs or until relapse, relapses occurred in 9% (13/142) attending the CSC, versus 3% (2/75) assessed in the field (p = 0.09). The rate for patients followed at CSC for ≥12 yrs and a pre-treatment BI ≥2.7+ was 13% (13/98). All relapses were BL or LL, with pre-treatment bacterial load influence relapse rates after 2 yr MDT.

RESUMÉ

Les estimations des taux de rechutes des patients lépreux multibacillaires (MB), après deux années de polychimiothérapie (PCT) selon les recommandations de l’OMS, varient. Entre 1987 et 1994, 500 MB patients hanséniens ayant complété deux années de PCT furent...
Dapsone monotherapy was used to treat patients with all forms of leprosy from the 1940’s until the 1990’s. However, concerns about dapsone resistance and relapses upon discontinuation of therapy, especially in those with multi-bacillary (MB) disease, led to the development of rifampin-containing bactericidal regimens in the 1970’s, modeled after successful short-course rifampin-based regimens for pulmonary tuberculosis (10). By 1982, the WHO abandoned dapsone monotherapy and introduced multiple-drug therapy (MDT), a convenient, relatively inexpensive regimen consisting of monthly rifampin and clofazimine, and daily dapsone and clofazimine administered for at least 2 yrs in MB patients. By 1994, MDT was implemented worldwide and the overall prevalence of leprosy, but not incidence, dropped dramatically because patients completing MDT were removed from prevalence lists (2, 17). Relapse rates after MDT were purported to be low, but sufficiently discrepant estimates, now coupled with evidence that...
patients with high pre-treatment *M. leprae* loads are at higher risk and that mean relapse incubation periods for rifampin-containing regimens are likely beyond 5 yrs, indicate a need for additional, rigorous, long-term studies to more clearly define the protective efficacy of 2 year MDT (1, 3, 9, 57, 58).

With the extraordinary time and effort required to treat and then follow patients to accurately measure relapse, coupled with widespread MDT implementation only since the early 1990’s, prospective, rigorously conducted studies are scarce (1, 3, 4, 6, 9, 57). Indeed, many reports are retrospective analyses expressed in absolute numbers, and lack adequate follow-up time or sufficient participants, or vary in the definition of relapse criteria, making comparisons across studies difficult (6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 18). For prospective 2 year MDT relapse studies, overall relapse rates range from 0% to 20%, likely reflecting variability in study site, treatment compliance, relapse definition, frequency and operational differences in follow-up, length of follow-up, and pre-treatment bacterial indices (BI) (1, 3, 5, 6, 8, 9, 11, 12, 13, 14, 15, 16, 19, 20, 21, 22, 23, 57).

Jamet found that post-MDT relapse rates increased from 3% to 20% when mean follow-up was extended from 3.5 to 6 yrs (9), and in 2 studies, the proportion of patients relapsing with high pre-treatment bacterial indices (BI; ≥4+) was notably higher (3, 9).

In 1987, we started a prospective longitudinal study to assess for relapses in MB patients who had satisfactorily completed 2 year MDT. Mean follow-up time was about 10 years per patient, longer than other similarly designed studies (1, 3, 9). Relapse was defined by 2 field expedient criteria, including new skin lesions consistent with leprosy and a BI increase of ≥2+ at any site (6, 9).

Relapse was defined as the appearance of new skin lesions consistent with leprosy and a BI increase of ≥2+ at any site (6, 9). Lesion tissue was then harvested for histology and mouse footpad inoculation, and the patient treated with ROM, consisting of 12 monthly, supervised doses of rifampin (600 mg), ofloxacin (400 mg), and minocycline (100 mg) (26). Lepra reactions, including reversal reaction (RR), characterized by swelling and erythema of existing leprosy lesions and erythema nodosum leprosum (ENL), characterized by crops of tender papulo-nodules, fever, and malaise, were graded as mild, moderate or severe (27). Reactions were treated with oral corticosteroids until resolution.

**Methods and Materials**

**Protocol.** Enrollment was conducted from 1987 to 1994 at the Cebu Skin Clinic (CSC), an established leprosy referral center for Cebu province. Any patient with MB leprosy who had satisfactorily completed 2 years of WHO-MDT and was willing to comply with long term follow-up was eligible. The cohort included patients treated and followed at the leprologist-staffed CSC, as well as those treated at local government clinics with a documented diagnosis of MB leprosy and satisfactory completion of 2 year WHO-MDT. MDT consisted of 24 observed doses of rifampin (600 mg) and clofazimine (300 mg) given at monthly intervals within a 24 to 30 month period, and unobserved daily dapsonse (100 mg) and clofazimine (50 mg), with no additional efforts to enhance self-administration (24).

Patients were excluded if they resided in a leprosaria or more than 100 kilometers away from the CSC, or had tuberculosis, malignancy, or other chronic systemic illness. Volunteers were advised on follow-up requirements and potential benefits, and a field team monitored some patients without transportation. All volunteers received vitamins and travel reimbursement.

To the greatest extent possible, clinical examinations, lepra reaction monitoring, and slit skin smears for acid-fast bacilli (AFB), following a standard 6 site sampling procedure (25), were done annually. Unless otherwise specified, all BI values refer to the mean value obtained from the 6 sites. Generally, patients treated at the CSC received follow-up by physician-leprologists every 1 to 2 years, and those recruited from the local government clinics received follow-up by a CSC field team every 2 to 3 years.

When more than 1 year had elapsed between smears for a relapsed patient, time to relapse was estimated by selecting the “midpoint” year of the period between most recent smear and the year of smear positivity (9).

Relapse was defined as the appearance of new skin lesions consistent with leprosy and a BI increase of ≥2+ at any site (6, 9). Lesion tissue was then harvested for histology and mouse footpad inoculation, and the patient treated with ROM, consisting of 12 monthly, supervised doses of rifampin (600 mg), ofloxacin (400 mg), and minocycline (100 mg) (26). Lepra reactions, including reversal reaction (RR), characterized by swelling and erythema of existing leprosy lesions and erythema nodosum leprosum (ENL), characterized by crops of tender papulo-nodules, fever, and malaise, were graded as mild, moderate or severe (27). Reactions were treated with oral corticosteroids until resolution.

**Histology and mouse footpad studies.** For each relapse, mouse footpad tests were
used to assess viability of *M. leprae* organisms and characterize drug sensitivities as previously described (28, 29, 30). A skin punch biopsy from a clinically active lesion was obtained and processed for inoculation into inbred CBA/J mice. Approximately $5 \times 10^3$ acid-fast bacilli (AFB) were inoculated into each hind footpad. One group of untreated mice was used to confirm *M. leprae* viability, whereas other groups of mice received clofazimine or dapsone in the feed (0.0001%, 0.001% and 0.01%), or rifampin twice weekly by gastric gavage (5 mg/kg, 10 mg/kg and 20 mg/kg). For all experiments, both hind footpads of each mouse were pooled, processed, stained (Fite-Faraco), and counted (28, 31).

*M. leprae* viability was determined by periodically sacrificing 1 or 2 untreated mice, starting at 6 months. Upon viability confirmation, defined as $\geq 1.5 \times 10^5$ *M. leprae*/footpad, treated mice were assessed for *M. leprae* growth. Drug resistance was defined as a harvest of $\geq 10^5$ *M. leprae*/footpad (20-fold increase in the inoculum).

**Data collection and analysis.** Data were recorded on standardized case report forms, entered into a computerized database (Excel 2000), and cross-checked for agreement. Graphical and statistical analyses were done by Sigmaplot 8 (SPSS, UK), SigmaStat (version 2.03, SPSS, UK), SPSS (UK), and Minitab (version 13). The primary outcome was relapse, and secondary outcomes were BI-relapse relationships, mouse footpad studies, and incidence of lepra reactions. Relapse rates were expressed as the percentage of patients relapsed, with incidence densities calculated as the number of relapsed patients divided by the total person-years of follow-up. The Kaplan-Meier method was used to estimate the cumulative risk of relapse during follow-up (32, 33). Lepra reactions were reported as proportions.

**RESULTS**

**Volunteers.** From 1987 to 1994, 500 patients were enrolled, including 184 government clinic referrals with documented satisfactory MDT completion. Recruitment and demographics are shown in Tables 1 and 2. Clinical classification was according to Ridley and Jopling (34). For bacterial loads, 181 volunteers had a “high” BI ($\geq 4.0+$) (36%) and 319 had a “low” BI (<4.0+) (64%). At MDT completion, 219 volunteers (44%) remained smear positive, with a downward trend through year 6, when all smears were negative. In general, BI values fell about 1 log per year. Through 2002, 83 patients were no longer in follow-up due to

<table>
<thead>
<tr>
<th>Demographic characteristics of volunteers.</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical diagnosis pre-MDT</strong></td>
<td>Mean BI</td>
<td>Mean BI at MDT completion</td>
</tr>
<tr>
<td>BT</td>
<td>24</td>
<td>1.3 (0.5–1.3)</td>
</tr>
<tr>
<td>BB</td>
<td>16</td>
<td>2.1 (0.8–2.3)</td>
</tr>
<tr>
<td>BL</td>
<td>253</td>
<td>2.9 (0.2–5)</td>
</tr>
<tr>
<td>LL</td>
<td>207</td>
<td>4.1 (1.2–5.5)</td>
</tr>
<tr>
<td>All</td>
<td>3.34 (0.2–5.5)</td>
<td>0.5 (0–4.3)</td>
</tr>
</tbody>
</table>

BT = borderline tuberculoid, BB = borderline, BL = borderline lepromatous, LL = lepromatous
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2003

Relapse (N = 15), death unrelated to leprosy (N = 32), ingestion of anti-leprosy drugs (N = 3), and relocation (N = 33). Overall, 465 volunteers remained in follow-up for ≥ 5 years or relapsed (Figure).

Relapses. Two volunteers were excluded from analysis because they failed to return for any follow-up. Through 2002, follow-up was 5368 patient-years, with a mean of 10.8 years per patient. Overall, the relapse rate was 3.0% (15/498), with an incidence density of 0.28/100 patient-years. A Kaplan-Meier survival curve (Figure) estimated cumulative risks of relapse at 5, 7, and 15 yrs of 0.64% (95% confidence interval [CI], 0.03%–1.25%), 1.7% (95% CI, 1.0%–2.4%), and 3.9% (95% CI, 2.2%–5.6%), respectively.

All relapses were borderline lepromatous (BL) or polar lepromatous (LL) before MDT and had pre-treatment BIs ≥ 2.7+. For the subset of patients followed at the CSC by physician-leprologists for ≥12 years, 13/142 (9%) relapsed vs. 2/75 (3%) followed in the field (p = 0.09, Fischer’s exact test). For a smaller group of patients within the CSC subgroup followed for ≥12 yrs and who had an initial BI ≥ 2.7+, the relapse rate was 13% (13/98) vs. 0% (0/44) in those with an initial BI <2.7 (p <0.001, Fisher’s exact test). There was no correlation between relapse incubation time and BI magnitude at relapse (r² = 0.26; p = 0.35).

Using midpoint estimates, relapses were recorded between 3 and 12 yrs, with a mean relapse incubation time of 7.9 yrs (95% CI, 6.4 to 9.3 yrs) (Table 3). Based on actual year of detection, relapses occurred between 6 and 13 yrs (7 relapses between 10 and 12 yrs), with a mean relapse incubation time 9.0 yrs (95% CI, 7.7 to 10.4 yrs).

All patients fulfilling the criteria for relapse presented with new papules, nodules, or plaques and a rising BI therein (5+ [N = 12], 4+ [N = 2], and 3+ [N = 1]). In all but a single relapse (R-341), the BI had become 0 at all 6 sites sampled on the examination prior to relapse detection. In 10 relapses, AFB were present in new skin lesions as well as earlobes, 8 bilaterally, with BIs ranging from 1+ to 5+ (mean 3.3+), whereas all other standard smear sites were negative for AFB. Overall, 5 relapsed volunteers had a BI of 0 on MDT completion, with all but one attaining a BI of 0 prior to relapse detection. For those with a high pre-MDT BI, 2 of 6 attained a BI of 0 at MDT completion.

Post-MDT reversal reaction (RR) and erythema nodosum leprosum (ENL), recorded only for volunteers recruited at CSC, were reported in 71 of 316 volunteers (23%), with 62 being graded as mild (Table 4) (27). The majority of RR (61%) and all ENL occurred within 5 years of MDT completion. Some volunteers developed multiple episodes of the same reaction type, but RR and ENL was never reported in the same patient. All reactions resolved with oral corticosteroids, without permanent sequelae.

Mouse footpad studies. Lesion samples from all relapses grew in untreated mice, confirming viability. All relapse isolates demonstrated sensitivity to all 3 tested rifampin and clofazimine dosage schedules. M. leprae from 12 relapsed patients were found to be fully dapsone sensitive. Relapse

### Table 2. Range of BI pre-MDT, and proportion of positive smears during and after MDT.*

<table>
<thead>
<tr>
<th>BI range</th>
<th>Number pre-MDT</th>
<th>Number at MDT completion</th>
<th>Number of smear positives yearly post-MDT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yr 1</td>
</tr>
<tr>
<td>5.0–6.0+</td>
<td>63</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.0–4.9+</td>
<td>118</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3.0–3.9+</td>
<td>142</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>2.0–2.9+</td>
<td>119</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>1.0–1.9+</td>
<td>48</td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td>0.1–0.9+</td>
<td>10</td>
<td>104</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>219/500 (43.8%)</td>
<td>42/202</td>
</tr>
</tbody>
</table>

* All smears negative at 6th year.
isolates R-499 and R-398 (Table 3) grew in mice treated with only the lowest concentration of dapsone (mild to negligible resistance), whereas isolate R-285 grew in mice treated with all 3 concentrations, compatible with “high” grade resistance.

**DISCUSSION**

Relapse was studied in a cohort of 500 MB leprosy patients treated reliably with 2 year MDT and followed prospectively by an experienced study site for a mean of 10.8 yrs, longer than most other studies (57). For a subset of 217 patients followed ≥12 yrs or until relapse, relapse rates were 9% (13/142) among those attending the leprologist-staffed CSC and 3% (2/75) assessed by a CSC field team, a notable difference suggesting that relapse detection may have been affected by medical expertise. Within a smaller subset of patients followed at CSC for ≥12 yrs and a pre-treatment BI ≥2.7, the relapse rate was 13% (13/98). In contrast, the relapse rate was 0% (0/44) in those followed for ≥12 yrs with pre-MDT BIs <2.7+ supporting contentions that higher pre-treatment BIs influence relapse risk (6). Overall, a total of 15 relapses yielded an absolute relapse rate of 3% (0.28/100 person-years) and a Kaplan-Meier survival analysis (adjusts for differences in follow-up) estimated the cumulative risk of relapse to be 3.9% at 15 yrs. Other notable findings included 8 of 15 relapses occurring within 7 years of MDT completion, and in agreement with other studies, most were in patients with high pre-MDT BI (≥4+) (57). Five of 15 relapses attained a BI of 0 by MDT completion, with all but one attaining a BI of 0 prior to relapse detection, emphasizing smear negativity is not necessarily protective against relapse (4). All relapses, fulfilling our 2 field-expedient relapse criteria of new skin lesions with an increased BI, were subsequently found to contain *M. leprae* that multiplied in mice, underscoring the reliability of our relapse definition. Especially encouraging was that none of the relapses were associated with peripheral neuropathy or disability. Post-
### Table 3. Clinical characteristics of relapses.

<table>
<thead>
<tr>
<th>Patient code</th>
<th>New skin lesions at relapse</th>
<th>Age at relapse</th>
<th>Pre-MDT diagnosis</th>
<th>Pre-MDT BI</th>
<th>Post-MDT BI</th>
<th>Year enrolled</th>
<th>Prior negative skin smear</th>
<th>Relapse smear after MDT***</th>
<th>Midpoint year to relapse skin smear after MDT**</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-341 M</td>
<td>Macules, papules, localized infiltration</td>
<td>43</td>
<td>BL</td>
<td>3.7</td>
<td>1.3</td>
<td>1987</td>
<td>1987</td>
<td>1993 (6)</td>
<td>1990 (3)</td>
</tr>
<tr>
<td>R-366 M</td>
<td>Macules, papules, localized infiltration</td>
<td>16</td>
<td>LL</td>
<td>5.0*</td>
<td>0.5</td>
<td>1992</td>
<td>1997</td>
<td>1999 (7)</td>
<td>1998 (6)</td>
</tr>
<tr>
<td>R-408 M</td>
<td>Nodules, localized infiltration</td>
<td>44</td>
<td>LL</td>
<td>4.5*</td>
<td>0.3</td>
<td>1993</td>
<td>1997</td>
<td>2001 (8)</td>
<td>1999 (6)</td>
</tr>
<tr>
<td>R-082 M</td>
<td>Localized infiltration</td>
<td>20</td>
<td>BL</td>
<td>4.0*</td>
<td>0</td>
<td>1991</td>
<td>1997</td>
<td>1998 (7)</td>
<td>1999 (7)</td>
</tr>
<tr>
<td>R-081 M</td>
<td>Macules, papules, localized infiltration</td>
<td>25</td>
<td>BL</td>
<td>2.7</td>
<td>0</td>
<td>1990</td>
<td>1997</td>
<td>2001 (11)</td>
<td>1999 (9)</td>
</tr>
<tr>
<td>R-080 M</td>
<td>Macules, papules, localized infiltration</td>
<td>39</td>
<td>LL</td>
<td>3.8</td>
<td>2.3</td>
<td>1987</td>
<td>1995</td>
<td>1997 (10)</td>
<td>1996 (9)</td>
</tr>
<tr>
<td>R-110 M</td>
<td>Localized infiltration</td>
<td>67</td>
<td>BL</td>
<td>3.7</td>
<td>1.8</td>
<td>1987</td>
<td>1994</td>
<td>1999 (12)</td>
<td>1997 (10)</td>
</tr>
<tr>
<td>R-161 M</td>
<td>Macules, localized infiltration</td>
<td>32</td>
<td>BL</td>
<td>2.7</td>
<td>0.7</td>
<td>1987</td>
<td>1997</td>
<td>1997 (10)</td>
<td>1997 (10)</td>
</tr>
<tr>
<td>R-398 M</td>
<td>Macules, localized infiltration</td>
<td>32</td>
<td>BL</td>
<td>4.0*</td>
<td>0</td>
<td>1989</td>
<td>2000</td>
<td>2001 (13)</td>
<td>2001 (12)</td>
</tr>
</tbody>
</table>

*pre-MDT “high” BI (≥4.0+)

** date (number of elapsed years)
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MDT reactions responded well to oral corticosteroids and resolved without permanent sequelae.

A notable observation was the proportional increase in relapse rates found in patients followed by physicians at CSC vs. those in the field. As care of leprosy patients is shifted to the general health service, wherein even rudimentary follow-up may not be feasible, much less long term follow-up, diagnosis of relapse MB leprosy may be delayed. This is problematic because early relapses, like here, may present with only new skin lesions. However, if relapses are detected only late, the risk for neurologic morbidity and deformity increases. Furthermore, since failure of MDT is generally confined to those with BL or LL and high BIs, the loss worldwide of the availability of skin smears and histopathology lessens the ability to identify those patients requiring the most rigorous follow-up. This argues for the maintenance and training of leprologists, emphasis on the importance of the diagnosis of LL and its relapse, and the re-establishment of skin smears and histopathology.

Recent reports suggest the relapse incubation periods after rifampin-containing regimens like MDT likely extend beyond a previously advocated range of 3 to 7 yrs (57). Indeed, Pattyn found that relapses after an intensive 6 week rifampin-containing regimen began at 6 yrs, but with a doubling in years 8 to 9 (58). Here, 7 of 15 relapses occurred at 9 or more years (midpoint estimate) after therapy, for an overall mean incubation period of 7.9 yrs. After characterizing 15 relapses through 2002, 3 others occurred in 2003, at years 13, 14, and 15 of follow-up, further underscoring the importance of extended follow-up for defining relapse rates. Notwithstanding, relapse incubation periods will invariably depend on relapse definitions, as would rates, in that a more rigorous definition might require longer follow-up to qualify as a relapse (3, 9, 57).

Irrespective of relapse definitions, it is impossible to know whether relapses reflect reactivation of the “persister” organisms that have survived MDT or re-infection (35, 36, 37, 38, 39, 40, 41, 42). Indeed, although all

<table>
<thead>
<tr>
<th>TABLE 4.</th>
<th>Lepra reactions after 2 year WHO-MDT.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction</td>
<td>Total*</td>
</tr>
<tr>
<td>RR</td>
<td>62/316 (20%)</td>
</tr>
<tr>
<td>ENL</td>
<td>9/316 (3%)</td>
</tr>
</tbody>
</table>

*There were no enrollees with both reversal reaction (RR) and erythema nodosum leprosum (ENL).

<table>
<thead>
<tr>
<th>TABLE 5.</th>
<th>Mouse footpad tests for viability and drug sensitivity.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient code</td>
<td>Control group</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Patient code</td>
<td>20 mg</td>
</tr>
<tr>
<td>R-341</td>
<td>16/16</td>
</tr>
<tr>
<td>R-158</td>
<td>7/7</td>
</tr>
<tr>
<td>R-499</td>
<td>14/14</td>
</tr>
<tr>
<td>R-285</td>
<td>10/10</td>
</tr>
<tr>
<td>R-162</td>
<td>14/14</td>
</tr>
<tr>
<td>R-161</td>
<td>2/2</td>
</tr>
<tr>
<td>R-082</td>
<td>8/8</td>
</tr>
<tr>
<td>R-110</td>
<td>10/10</td>
</tr>
<tr>
<td>R-207</td>
<td>6/6</td>
</tr>
<tr>
<td>R-081</td>
<td>2/2</td>
</tr>
<tr>
<td>R-366</td>
<td>6/6</td>
</tr>
<tr>
<td>R-422</td>
<td>13/13</td>
</tr>
<tr>
<td>R-053</td>
<td>7/7</td>
</tr>
<tr>
<td>R-408</td>
<td>5/5</td>
</tr>
<tr>
<td>R-398</td>
<td>2/2</td>
</tr>
</tbody>
</table>

*low dapsone resistance, **high dapsone resistance, NH (no harvest)
relapses were essentially sensitive to the MDT components, arguing against the development of drug resistance, murine monitoring of several rifampin-based regimens for MB leprosy indicate that “persisters” surviving rifampin therapy regularly occur in 9% of MB patients (43), coinciding with the 9% relapse rate among those with ≥12 yrs, CSC physician-based follow-up. In tuberculosis (TB), RNA restriction fragment length polymorphisms show that in AIDS populations, almost half the infections are new, whereas in populations with low endemicity, the majority are reactivation (44, 45). Genotypic assays that detect differences among M. leprae strains may help to distinguish reactivation from reinfection in leprosy, as well as whether certain M. leprae strains predispose to relapse and which biochemical anomalies confer treatment failure (46, 47, 48).

An important goal of MDT is the prevention of multiple drug-resistant M. leprae. Here, relapse M. leprae was uniformly sensitive to rifampin and clofazimine, and only 3 (20%) isolates showed dapsone resistance (2 low level, 1 fully resistant), surprising in that up to 52% of our untreated patients in the early 1990’s harbored primary dapsone resistant M. leprae (low, moderate and high) (28). Clearly, our current data argue that 2 year MDT prevented the emergence of drug-resistant M. leprae, paralleling observations in effective multi-drug TB regimens (10).

In this study, even within subsets of patients suggesting that the relapse rate may be as high as 9% or even 13%, MDT provided a reasonably high cure rate. In contrast, other prospective relapse studies report rates of up to 20%, with even higher rates among patients with a high pre-MDT BI, usually characterized by a still positive BI after MDT completion (9, 14, 19, 49, 57). In particular, the Marchoux Study Group (Mali) reported relapses in 20% (7/35) of MB patients with a mean follow-up of 6 years, but a rate of 39% for those with a pre-treatment BI ≥4+ (6). Girdhar (India) reported relapses in 7% (20/260) of MB patients with a mean follow-up of 4 years, with a 17% (18/170) rate among those with a pre-treatment BI ≥4+ versus 1% (2/153) with a BI <4+ (7). Late relapses here and in Pattyn’s study, however, suggest that relapse rates after 2 year MDT in Mali and Agra may have increased with additional follow-up (50).

In 1998, the WHO reduced the duration of MDT to 1 year for MB patients, with an eventual goal of 6 months for all forms of leprosy and a cumulative relapse rate of ≤5% at 5 yrs, paralleling accepted rates for pulmonary TB (41). Previously, relapse rates greater than 5% after therapy of pulmonary TB were rejected by the British Medical Research Council as inadequate (10), and recent findings of a 9% relapse rate after 6 months therapy for cavitary TB (51), like MB leprosy with a high bacterial burden, prompted calls to extend therapy to 9 months (52). Analogously, our MDT relapse data, along with others, might argue that even 2 year MDT is inadequate in MB leprosy, especially for patients with high BI before treatment, and moreover, that 5 years of follow-up is insufficient to accurately define relapse risks.

Alternatives to 2 year MDT include: (i) the proven modality of lifelong dapsone therapy after 5 years of daily dapsone and rifampin (55), (ii) extending MDT, or (iii) highly bactericidal combinations that include rifampin or ofloxacin (or congeners), clarithromycin, or minocycline (54). Indeed, in the Agra study, the 17% relapse rate found in MB patients treated for 2 years with an initial BI ≥4 was reduced to 4% when MDT was extended until smear negativity, an average of 5 years on treatment (5). According to observations in mice whereby minocycline alone or in combination with moxifloxacin added to the bactericidal activity of rifamycin (54, 55), and in the highly baciliferous and immunosuppressed neonatally thymectomized Lewis rat whereby minocycline + rifampin, but not rifampin alone, consistently eliminated all viable M. leprae (56), minocycline and perhaps moxifloxacin may be especially beneficial components to any future rifamycin-containing regimen.

Acknowledgment. This study was supported by grants from the World Health Organization (UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases), Pacific Leprosy Trust (New Zealand) and the Sasakawa Memorial Health Foundation of Japan. Rico Abella, Jay Vicada, Junie Abellana, Mary Grace Bordon, Mercedita Hinaloc, and the LWM laboratory staff contributed to this work. Guillerma Lim and Pris Reed provided administrative support.
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Nail Involvement in Leprosy: A Study of 300 Patients

Inderjeet Kaur, Aditi Chakrabarti, Sunil Dogra, Ranju Rai, and Bhushan Kumar

ABSTRACT

Three hundred leprosy patients were recruited to study the pattern and frequency of nail changes. Nail changes, like longitudinal ridging in finger nails, transverse striations involving both finger and toe nails etc. which occurred with similar frequency in the PB and MB patients in comparison with the control group, were excluded from the analysis. Out of a total number of 150 PB patients, 84 (56%) showed nail changes. Fifty-eight (38.6%) patients showed changes in the finger nails, with an average of 3.2 involved nails per patient. Fifty-three (35.3%) patients showed changes in the toe nails, with an average of 3.0 nails per patient. The most common change observed was longitudinal melanonychia (32.4%) in the finger nails and longitudinal ridging (46.3%) in the toe nails.

In comparison, 131/150 (87.3%) MB patients showed nail changes. Finger nail changes were seen in 102 (68%) patients with an average of 5.5 nails affected per patient. Changes in toe nails were seen in 116 (77.3%) patients, with an average of 6.0 nails involved per patient. The most common nail change observed was longitudinal melanonychia in 89/523, (17%) of the total involved finger nails and subungual hyperkeratosis in 164/702, (23.4%) of the total toe nails involvement. Out of a total of 32 colony patients, 31 (96.9%) showed nail changes both in finger and toe nails with an average of 7.9 and 8.4 affected nails per patient, respectively. The most common nail change observed was rudimentary nail(s) on fingers (29%) and toes (21.1%). Among MB patients, a significantly higher number had finger nail involvement in LL group. The frequency of nail involvement for both fingers and toes was significantly greater in LL as compared to BL group of patients. The frequency of nail involvement was significantly more in patients having disease for more than 5 years and in those having trophic changes secondary to loss of sensations and impaired circulation.

RESUMÉ

Trois cent patients lépreux furent recrutés afin d’étudier la fréquence et les aspects de lésions des ongles. Les changements observés sur les ongles, qui apparaissent avec une fréquence similaire entre les patients PB et MB comparé au groupe témoin, comme les stries longitudinales sur les ongles de doigts de la main, les striations transversales sur les ongles de main et de pied, etc., furent exclus de l’analyse. Parmi un total de 150 patients PB, 84 (56%) ont montré des lésions des ongles. Cinquante huit (38,6%) de ces patients ont montré des lésions des ongles des mains, avec une moyenne de 3,2 ongles atteints par malade. Cinquante trois (35,3%) de ces patients ont montré des lésions des ongles des pieds, avec une moyenne de 3,0 ongles atteints par patient. La lésion la plus fréquente était la mélanonychie longitudinale (32,4%) pour les ongles des mains et les irrégularités longitudinales (46,3) pour les ongles des pieds.

Par comparaison, 131/150 (87,3%) des patients MB ont montré des lésions unguéales. Les altérations des ongles des mains furent observées chez 102 (68%) malades, avec une moyenne de 5,5 ongles affectés par patient. Les lésions unguéales des pieds furent détectées chez 116 (77,3%) patients, avec une moyenne de 6,0 ongles atteints par patient. La lésion la plus commune observée était la mélanonychie chez 89/523 (17%) des ongles atteints des mains et l’hyperkératose sub-inguéale chez 164/702 (24,4%) des ongles de pied atteints. Parmi un nombre total de 32 patients relatifs entre eux, 31 (96,9%) ont montré des lésions unguéales à la fois des mains et des pieds, avec une moyenne de 7,9 et 8,4 ongles af-
Leprosy is a chronic infectious disease affecting almost every organ of the body with wide ranging clinical manifestations. All organs and systems involved have been studied quite extensively in leprosy, but the involvement of nails has never been fully highlighted. Although the dystrophic changes and mutilation of hands and feet are considered more or less synonymous with the symptomatology of the disease, nail changes have received only a passing reference in the literature (5, 9).

Nail changes can be caused by neuropathy, repeated trauma, vascular deficit, infections and often more than one factor is involved. There have been only few case reports describing isolated nail changes in leprosy patients (7, 14, 19). In the only published study on large number of leprosy patients, the incidence of nail changes was found to be 64% (15). It was observed that despite wide differences in pathology, nail changes in tuberculoid and lepromatous patients were often similar and that lepromatous patients developed bilaterally symmetrical nail changes late in the course of the disease. However, there was a lack of systematic categorization and detailed description of the pattern of nail changes observed. Scanty data on the subject prompted us to study the frequency and pattern of nail changes and their correlation with the type of disease, duration of disease, deformity of hands/feet, and treatment status in patients with leprosy attending our leprosy clinic and also in patients residing in a leprosy colony, regardless of the duration of disease or treatment status.

MATERIALS AND METHODS

A total of 300 patients, 150 consecutive patients each with paucibacillary (PB) and
multibacillary (MB) leprosy in the age group 20–50 yrs irrespective of sex, duration of disease, treatment, and reactional status attending the leprosy clinic at Postgraduate institute of Medical Education and Research, Chandigarh, India were included in the study (Table 1). One hundred age and sex matched control subjects not suffering from any disease known to affect the nails and 32 treated patients of leprosy residing in a nearby leprosy colony were also recruited in the study. Patients were diagnosed according to Ridley-Jopling classification and grouped into into PB (polar tuberculoid, TT, and borderline tuberculoid, BT) and MB (borderline, BB; borderline lepromatous, BL; polar lepromatous, LL) disease for purposes of analyses. Histopathological study of the skin lesions and slit skin smear examination were done as a routine in all patients. In addition to the routine cutaneous and neurological examination, various nail changes were noted in a predesigned proforma taking into account the number and distribution of (fingers/toes) nails involved. Peripheral vascular status of the extremities was evaluated clinically by palpating the major arterial pulsations of both upper and lower limbs. Deformities of the hands and feet were recorded according to the WHO grading (1988) (20). Fungal infections of the nails were ruled out by KOH examination and culture whenever indicated. X-rays of the hands and/or feet were done only in a limited number of cases. The pattern of nail changes in the study and control groups were compared using chi-square test. The following nail changes, which occurred with similar frequency in the leprosy patients (both PB and MB patients) and the control group, were excluded from the analysis: i) longitudinal ridging and flattening—in finger nails; ii) longitudinal splitting, transverse pigmented bands, and blackish discoloration—in toe nails; iii) absence of lunula, leukonychia, transverse striations, rough nails, loss of nail fold and pitting—in both finger and toe nails. Various noted nail

<table>
<thead>
<tr>
<th>Table 1. Frequency of nail involvement in paucibacillary (PB) and multibacillary (MB) patients.</th>
</tr>
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<tbody>
<tr>
<td>PB cases (N = 150)</td>
</tr>
<tr>
<td>BT cases (N = 150)</td>
</tr>
<tr>
<td>No. of patients affected</td>
</tr>
<tr>
<td>No. of nails affected</td>
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<tr>
<td>No. of affected nails/patient</td>
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<table>
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<tr>
<th>Table 2. Pattern of nail changes in PB patients.</th>
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<tbody>
<tr>
<td>Nail changes</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Longitudinal melanonychia</td>
</tr>
<tr>
<td>Subungual hyperkeratosis</td>
</tr>
<tr>
<td>Longitudinal ridging</td>
</tr>
<tr>
<td>Beau’s line</td>
</tr>
<tr>
<td>Flattening</td>
</tr>
<tr>
<td>Onycholysis</td>
</tr>
<tr>
<td>Thickening of nail plate</td>
</tr>
<tr>
<td>Reddish brown discoloration</td>
</tr>
<tr>
<td>Thinning of nail plate</td>
</tr>
<tr>
<td>Onychomycosis</td>
</tr>
<tr>
<td>Longitudinal splitting</td>
</tr>
<tr>
<td>Chronic paronychia</td>
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<tr>
<td>Pterygium unguium</td>
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</tbody>
</table>

* Column percentage.
findings were classified based on changes in morphology and color, involving different components of the nail unit like nail plate, nail bed, nail folds viz. longitudinal melanonychia, excessive curvature of nail plate, subungual hyperkeratosis, rudimentary nails, etc. as given in Tables 2 and 3.

**RESULTS**

**Paucibacillary patients.** In the PB group, all 150 patients had BT disease. There were 106 (70.7%) males and 44 (29.3%) females. The mean age of male and female patients was 32.8 ± 2.1 and 28.1 ± 3.2 yrs, respectively. Of these, 111 patients were untreated and the rest were on regular World Health Organization (WHO) Multidrug therapy (MDT) PB regimen or had completed the treatment. The duration of the disease in these patients ranged from 1 month to 13 yrs (mean 13 ± 4.1 months). Thirty-four patients were in type I reaction. Duration of the reaction ranged from 20 days to 1 yr (mean 4.7 ± 2.9 months). Twenty-six patients had deformities of hands (grade I in 10 and grade II in 16), 10 had deformities of feet (grade I in 7 and grade II in 3) and 2 patients had deformities of both hands and feet (grade 1).

Out of these 150 PB patients, 84 (56%) showed nail changes. Fifty-eight (38.6%) patients showed changes in the finger nails, with an average of 3.2 nails per patient. Fifty-three (35.3%) patients showed changes in the toe nails, with an average of 3.0 nails per patient (Table 1). Sixty-one (40.7%) patients had involvement of both finger and toe nails. Involvement of either fingernails or toe nails alone was seen in 15 (10%) and 8 (5.3%) patients, respectively.

The most common nail change observed was longitudinal melanonychia (one to three bands) in the finger nails (32.4%) (Fig. 1) and longitudinal ridging (46.3%) in the toe nails (Table 2). Various types of nail changes and their frequencies are given in Table 2. The presence of nail changes was significantly greater in those having the disease for a period of more than 5 years (55/84 vs. 23/66) (p <0.05). The pattern of nail changes did not differ significantly with an increase in the duration of the disease beyond 5 years. Nail changes were not found to be more frequent in relation to lep-

<table>
<thead>
<tr>
<th>Nail changes</th>
<th>No. of patients</th>
<th>Total no. of nails involved</th>
<th>Fingers (%)*</th>
<th>Toes (%)*</th>
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<tbody>
<tr>
<td>Subungual hyperkeratosis</td>
<td>71</td>
<td>24</td>
<td>47</td>
<td>57 (10.1)</td>
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<tr>
<td>Logitudinal ridging</td>
<td>43</td>
<td>—</td>
<td>43</td>
<td>—</td>
</tr>
<tr>
<td>Complete shedding</td>
<td>41</td>
<td>20</td>
<td>21</td>
<td>63 (11.2)</td>
</tr>
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<td>Onychauxis</td>
<td>37</td>
<td>14</td>
<td>23</td>
<td>17 (3.0)</td>
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<td>35</td>
<td>35</td>
<td>—</td>
<td>89 (15.8)</td>
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<tr>
<td>Rudimentary nails</td>
<td>32</td>
<td>15</td>
<td>17</td>
<td>55 (9.8)</td>
</tr>
<tr>
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<td>29</td>
<td>12</td>
<td>17</td>
<td>21 (3.7)</td>
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<tr>
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<td>25</td>
<td>19</td>
<td>6</td>
<td>46 (8.2)</td>
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<td>12 (2.1)</td>
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<td>17</td>
<td>6</td>
<td>11</td>
<td>19 (3.4)</td>
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<tr>
<td>Excessive curvature</td>
<td>14</td>
<td>11</td>
<td>3</td>
<td>38 (6.8)</td>
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<td>Pallor</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>49 (8.7)</td>
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<td>8</td>
<td>3</td>
<td>5</td>
<td>7 (1.2)</td>
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<tr>
<td>Fingering</td>
<td>7</td>
<td>—</td>
<td>7</td>
<td>—</td>
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<tr>
<td>Onychogryphosis</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>5 (0.9)</td>
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<tr>
<td>Brachytelephalangia</td>
<td>7</td>
<td>4</td>
<td>3</td>
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</tr>
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<td>Longitudinal splitting</td>
<td>5</td>
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<td>—</td>
<td>8 (1.4)</td>
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<td>reddish brown discoloration</td>
<td>4</td>
<td>4</td>
<td>—</td>
<td>24 (4.2)</td>
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<tr>
<td>Brittle nail</td>
<td>3</td>
<td>—</td>
<td>3</td>
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<tr>
<td>Pterygium unguium</td>
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<td>1</td>
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<tr>
<td>Central anonychia with polynychia</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>2 (0.3)</td>
</tr>
</tbody>
</table>

*Column percentage
rosy lesions on hands or feet, nor was any particular pattern found to correlate with the presence of these lesions. Nail changes such as nail dystrophy (Fig. 2), onycholysis, longitudinal ridging and brittle nails were observed to be more frequent in digits with sensorimotor deficit (p <0.01). Onychomycosis confirmed by potassium hydroxide (KOH) and/or culture was present in 6 (4%) patients with or without nail changes of leprosy.

**Multibacillary patients.** In the MB group (BL-60, LL-90), there were 96 (64%) males and 54 (36%) females with a mean age of 34 ± 2.1 yrs and 31 ± 1.4 yrs, respectively. Duration of the disease varied from 8 months to 19 yrs (mean 3.4 ± 1.5 yrs). Eighteen patients had type 1 reaction with duration of 1 week to 9 months (mean 4.25 ± 1.2 months) and 21 patients had type 2 reaction (including recurrent episodes) with duration of 3 months to 2 yrs (mean 12.7 ± 2.1 months). Ninety-six patients had deformities of the hand (grade I in 44, grade II in 52) and 104 patients had deformities of the feet (grade I in 56, grade II in 48).

Out of a total of 150 MB patients, 131 (87.3%) showed nail changes. Finger nail changes were seen in 102 (68%) patients with an average of 5.5 nails per patient (Table 1). Toe nail changes were seen in 116 (77.3%) patients, with an average of 6.0 nails per patient. One hundred seven (71.3%) patients had changes in both finger nails as well as toe nails. Seven (4.6%) patients had changes only in the finger nails while 17 (11.3%) showed changes in the toe nails alone. The number of patients having finger nail involvement was significantly more in the LL group than BL group (p <0.05). However, for toe nail involvement, this difference was not found to be statistically significant (p >0.1). The frequency of nail involvement for both fingers and toes was significantly more in the LL as compared to the BL group of patients (p <0.05) (Table 1). The most common nail change observed was longitudinal melanonychia (up to five bands) in the finger nails (15.8%) and subungual hyperkeratosis (23.4%) (Fig. 3) in the toe nails (Table 3). The frequency of nail changes was significantly greater in those having neurovascular deficit in the extremities (p <0.01). Certain patterns, like over curvature of nails (Fig. 4), complete shedding, rudimentary nails (Fig. 5) in both fingers and toes, and blackish discoloration of the finger nails were significantly more in those having the disease for a period of greater than 5 years and those having grade II deformities of the hands and feet (p <0.05). Nail changes like nail dystrophy, onycholysis, longitudinal ridging and brittle nails were observed to be more frequent in limbs with trophic changes secondary to loss of sensations and impaired circulation (p <0.01). Onychomycosis confirmed by KOH and or culture was present in 8 (5.3%) patients with or without nail changes of leprosy.

**Patients from leprosy colony.** There were 32 patients residing in the leprosy colony. The total duration of the disease
was more than 10 years in all of them. All of them had received adequate MDT or dapsone monotherapy and were smear negative. Thirty-one (96.9%) patients showed changes in both finger and toe nails, with involvement on an average of 7.9 finger nails per patient and an average of 8.4 toe nails per patient, respectively. The most common nail change observed was rudimentary nail(s) on fingers (29%) and toes (21.1%).

**DISCUSSION**

Nails are specialized keratinous cutaneous appendages that play an important role in the everyday life of humans, and have always provided the clinician major clues in the diagnosis of a number of diseases. The nail unit responds to a wide variety of insults by a limited number of reaction patterns. In literature, there is a paucity of comprehensive studies on the frequency and pattern of nail changes in leprosy patients. Bryceson and Pfaltzgraf (5) and Jopling (9) first described the nail changes in advanced lepromatous leprosy. Patki and Baran (15) reported nail changes in different spectrum of leprosy, but these studies lack information about the pattern of nail changes and their frequency. Nail changes in our patients were noted more frequently in the MB (87.3%) patients than in those with PB disease (56%). This could be attributed to the extensive bilateral peripheral neuropathy, trauma, infections, more severe degree of deformities and repeated type 2 reactions leading to immunologically mediated vasculopathy in the MB cases. In another published series, Patki and Baran (15) reported the incidence of nail changes to be 64% in their patients (PB/MB). Nail changes mostly occur as a result of nerve involvement. Like leprosy, neuropathy is a part of diabetes mellitus in which various nail changes like dystrophy, shortening, fragility, and yellowish nail discoloration have been described (1, 12, 17). There are reports of dystrophic changes in little finger nail following traumatic ulnar nerve damage (13, 17). In addition, the misuse and disuse of anesthetic and deformed hands and feet results in repeated mechanical and thermal trauma, infection, osteolysis of phalanges, and loss of nails. Trauma related changes like onycholysis, Beau’s lines, longitudinal ridging and splitting were more common in MB patients in our study, probably because anaesthetic but functional limbs in MB cases are more frequently traumatized due to misuse.

The trophic changes of leprosy have always been attributed mostly to the nerve damage, but it seems that the vascular component is also important, since such dys-
trophic nail changes are also seen in other diseases like scleroderma, rheumatoid arthritis etc, where vascular factors play an important role (10, 17, 18). The associations between neurovascular deficit and various trophic changes including fungal infections have been well documented in the literature (1, 8). Longitudinal ridging of the toe nails was a common change in both PB and MB patients. Similar changes have been described with peripheral vascular deficit and also as an age-related change (17, 18), possibly a result of age-related circulatory compromise. Other changes attributable to vascular deficit, like onycholysis, longitudinal splitting and brittle nails were seen more often in the MB patients. Beau’s lines have been reported to be associated with type 2 leprosy reactions and vasculitis was thought to be a probable cause. Since only few of our patients were in reaction at the time of study, we did not try to correlate any nail change with reactional status of the patient.

Longitudinal melanonychia was a common change observed in 33/150 (22%) and 35/150 (23.3%) of our PB and MB cases, respectively, and in 21.9% patients from the leprosy colony. According to Baran (1), these bands arise due to stimulation of melanocytes in the nail matrix following repeated trauma. Subungual hyperkeratosis was another notable change observed more commonly in the MB as compared to the PB patients. Though the exact explanation is not known, this has been speculated to be the adverse effect of clofazimine therapy given in anti-inflammatory doses (7). Pallor of the nails noted by us in both PB and MB patients, though not being specific for the disease, can probably be attributed to anemia due to the anti-leprosy drugs, notably dapsone and partly due to disease itself and also vascular deficit secondary to vessels involvement. Similar observations were made earlier by Patki and Baran (15).

Complete shedding of the nails, brachytelephalangia, and rudimentary nails with loss of terminal phalanges were the changes found exclusively in MB patients and in patients residing in the leprosy colony. This is consistent with the hypothesis of Baran and Juhlin (2) which states that development of a normal nail is dependent on the underlying bone; anonychia or hyponychia may result when the underlying bone is either hypoplastic or completely absent. In leprosy it is possible that nails may be affected secondary to resorption of distal phalanges. This was probably why anonychia and rudimentary nails were more common in patients from the leprosy colony whose fingers and toes showed marked bony resorption. Brand (4) confirmed with follow-up radiographs over a period of 5 years that approximately 95% of resorption resulted from open wounds developing a secondary infection.
Pardo-Castello and Pardo (13) noted tinea unguium in 32% of their leprosy patients. Ramesh and Misra (16) have postulated that nails could be the source of frequent tinea corporis and tinea cruris infections in leprosy patients. We noted onychomycosis in only 4.7% of our patients. Patki and Baran (15) postulated that in contrast to frequent dermatophytic infections, candidial paronychia is not often observed in leprosy patients who generally have dry skin (unless altered by their occupation), which is unsuitable for growth of *Candida albicans*. We noted only 5 cases of chronic paronychia, none of which were of candidial etiology. In our bid to find out any relationship between the nail changes and anaesthetic leprosy lesions located on the corresponding hands or feet, we could not establish any such association. Diffuse leukonychia or pseudomacrolunula, which was described by Pardo Castello and Pardo (13) as an early change in leprosy, was not noted by us. We only observed punctate leukonychia in our patients and its incidence was no more than in controls. In our opinion, since loss of nails in leprosy is a consequence of various interacting factors like vasculopathy, neuropathy and their sequelae like trauma and infection, the term shedding should be replaced by “acquired anonychia” to describe nail dystrophy and loss. Central anonychia with polyonychia was seen in one of our MB patients from the clinic and 3 patients from the leprosy colony. This change has been characterized described in congenital onychodysplasia of the index fingers (COIF) (11).

From our study it can be concluded that nail changes are common in leprosy, more so in the MB spectrum. Attributing a particular change to a single pathophysiologic mechanism would be an oversimplification. Peripheral neuropathy, trauma, and vasculopathy and secondary infections all play a role in the genesis of these nail changes. Several changes have been found in impressive numbers, but whether all are specific for the disease will remain speculative unless a clinicopathological study correlates our observations.

REFERENCES

Ultrastructural Study of Schwann Cells and Endothelial Cells in the Pathogenesis of Leprous Neuropathy

V. Kumar and U. Sengupta

ABSTRACT

Peripheral nerve biopsies from 4 borderline tuberculoid (BT) and 4 lepromatous (LL) patients who were on multidrug therapy were investigated by light and electron microscopic studies. The variation of diameters and distribution of myelinated and unmyelinated fibers between BT and LL patients were not significant. This study has shown significant changes in peripheral nerves and endoneural blood vessels. It was revealed that besides Schwann cells (SC), the endothelial cells (EC) of endoneural blood vessels frequently harbor M. leprae. In BT, peripheral nerves in addition to the degenerative changes of SC and presence of perineural and perivascular cuffing by mononuclear cells, the endoneural blood vessels showed thickening of basement membrane with hypertrophy of EC leading to narrowing or complete occlusion of lumen. On the other hand, peripheral nerves of LL patients were infiltrated with large number of M. leprae shown to be present in the electron transparent zone (ETZ) of the SC. The EC of endoneural blood vessels were found to be loaded with M. leprae, and this bacillary loaded EC was found to release M. leprae into the lumen through its ruptured membrane.

RESUMÉ

Les biopsies de nerfs périphériques provenant de 4 patients tuberculoïdes borderlines (BT) et de 4 patients lépromateux (LL) soumis à une polychimiothérapie (PCT) ont été étudiées par microscopies optique et électronique. Aucune différence significative fut observée en terme de diamètre et de distribution des fibres myélinisées et non myélinisées entre les patients BT et LL. L’étude a montré des différences significatives dans les nerfs périphériques en particulier au sein des vaisseaux sanguins intra-neuraux. Il a été mis en évidence que, en plus des cellules de Schwann (CS), les cellules endothéliales (CE) des vaisseaux intra-neuraux hébergaient fréquemment des M. leprae. Au sein des nerfs périphériques des patients BT, en plus des lésions dégénératives des CS et de la présence de manchons pérvasculaires de cellules mononucléées, les vaisseaux sanguins intra-neuraux montraient un épaissement des membranes basales associées à une hypertrophie des CE, résultant en un rétrécissement voire une occlusion complète de la lumière. D’autre part, les nerfs périphériques des patients LL étaient infiltrés par un grand nombre de M. leprae, localisées dans les CS et les ETZ ; les CE des vaisseaux sanguins intra-neuraux étaient remplies de M. leprae, et certaines de ces CE surchargées montraient parfois un relarguage de M. leprae dans la lumière au travers de la membrane cellulaire rompue.

RESUMEN

Se analizaron, por microscopía de luz y electrónica, las biopsias de nervios periféricos de 4 pacientes con lepra tuberculoiide subpolar (BT) y 4 pacientes lepromatosos (LL), que estuvieron en tratamiento con poliquimioterapia. Las variaciones en el diámetro y distribución de las fibras mielinizadas y no mielinizadas entre los pacientes BT y LL no fueron significativas. Sin embargo, el estudio mostró cambios importantes en los nervios periféricos y en los vasos sanguíneos endoneurales. Se observó que además de las células de Schwann (CS), las células endoteliales (CE) de los vasos sanguíneos endoneurales con frecuencia también contienen M. leprae. En la lepra BT, los nervios periféricos mostraron cambios degenerativos de las CS con acumulación perineural y perivascular de células mononucleares,
Leprosy is a chronic disease, caused by *Mycobacterium leprae* where involvement and damage of peripheral nerves is a typical and unique feature. One of the cardinal signs of clinical diagnosis of leprosy patients depends on the recognition of thickened peripheral nerves in patients supplying an anesthetic area in the skin, hand, legs, or face. The histopathological demonstration of *M. leprae* in the nerve and the presence of inflammatory granuloma in and around a nerve are mandatory for confirmation of diagnosis. However, the bacilli have not yet been reported in the brain and spinal cord, possibly due to the unfavorable condition for survival and growth of *M. leprae* in these areas (1). The disease is manifested in a spectrum based on the cell mediated immunity (CMI) of the host. While a strong CMI against *M. leprae*, which limits the growth of the bacilli, is expressed in tuberculoid (TT), and borderline tuberculoid (BT) leprosy, in lepromatous leprosy (LL) there is a lack CMI against the bacilli leading to unlimited growth of *M. leprae* in the host. In the early stage of the disease, even when only limited numbers of skin lesions are present and only small number of *M. leprae* are found in the skin, the organisms preferentially localize in the peripheral nerves.

Histologically, bacilli are seen within cells, either in myelinating or nonmyelinating Schwann Cells (SC) of nerves or in macrophages (7, 17, 18, 14). Presence of *M. leprae* has been shown by electron microscopy in SC of myelinated (MY) and unmyelinated (UMY) nerve fibers and also in macrophages, endothelial cells (EC), and perineurial cells (23, 12, 8, 9). Ultrastructurally, *M. leprae* have been found in the EC of blood vessels (5, 2, 3, 21, 19). It has been clear therefore, that the SC are not the only target cells for *M. leprae* growth, but organisms also grow in the EC of blood vessels. Recently, in an experimental model *M. leprae* has been observed in the EC of epineurial and perineurial blood vessels and also in lymphatics (28, 27). However, it is known that the endoneurial blood vessels supply the nutrients to the nerves for maintaining the metabolic activity of SC and is essential for proper nerve fiber functioning. Therefore, parasitization of the EC of endoneurial blood vessels could be also an essential feature for initiation of nerve damage.

The present study has been carried out to record the detailed ultrastructural changes in the SC and EC of endoneurial blood vessels which might help in understanding the morphological significance of pathogenesis and dissemination of *M. leprae*.

**MATERIALS AND METHODS**

**Nerve biopsies.** Eight patients of leprosy (4 BT and 4 LL) who volunteered for biopsy of the peripheral nerves were included in this study. The patients were selected from the out patient clinic of the

### The Table. Details of selected patients.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Type of disease</th>
<th>Age in years</th>
<th>Duration of the disease</th>
<th>Status of nerves</th>
<th>Nerve biopsied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BT</td>
<td>23</td>
<td>3 months</td>
<td>Thickened</td>
<td>Medial cutaneous</td>
</tr>
<tr>
<td>2</td>
<td>BT</td>
<td>27</td>
<td>6 months</td>
<td>Thickened</td>
<td>Superficial peroneal</td>
</tr>
<tr>
<td>3</td>
<td>BT</td>
<td>38</td>
<td>8 months</td>
<td>Thickened</td>
<td>Infra patellar</td>
</tr>
<tr>
<td>4</td>
<td>BT</td>
<td>25</td>
<td>8 months</td>
<td>Thickened</td>
<td>Medial cutaneous</td>
</tr>
<tr>
<td>5</td>
<td>LL</td>
<td>47</td>
<td>1 year</td>
<td>Thickened</td>
<td>Superficial peroneal</td>
</tr>
<tr>
<td>6</td>
<td>LL</td>
<td>36</td>
<td>1 year</td>
<td>Thickened</td>
<td>Infra patellar</td>
</tr>
<tr>
<td>7</td>
<td>LL</td>
<td>48</td>
<td>1 year 6 months</td>
<td>Thickened</td>
<td>Medial cutaneous</td>
</tr>
<tr>
<td>8</td>
<td>LL</td>
<td>52</td>
<td>2 years</td>
<td>Thickened</td>
<td>Superficial peroneal</td>
</tr>
</tbody>
</table>
Central JALMA Institute for Leprosy, Agra. Peripheral nerves from all of these patients were biopsied by employing standard surgical procedures (Table 1). Before conducting the study an ethical clearance was obtained from the Institutional Ethical Committee.

One part of the biopsy was fixed in formaldehyde and processed for histopathology. The other part was fixed in 2.5% glutaraldehyde for electron microscopic studies.

**Tissue preparation for electron microscopy.** Small pieces of the nerve biopsies were fixed overnight in 2.5% glutaraldehyde (TAAB Laboratory equipment, U.K.). Subsequently, the tissues were washed in phosphate buffered saline (PBS), (Itron Laboratory Inc., Japan) post fixed in 1% osmium tetroxide (OsO₄) (John Matthey Chemical, U.K.) and again washed in PBS. The fixed tissues were then dehydrated in ascending grades of alcohol. Later, these were immersed in propylene oxide (Fluka AG, Chemische Fabrik, Switzerland) and embedded in Spurr’s resin (TAAB Laboratories Equipment, England). Finally, the tissues were polymerized overnight at 70°C and blocks were made. Cross sections, 1 µ
FIGS 3–4. 3a, Ultrathin sections of peripheral nerve from BT showing Schwann cells with well preserved collagen fibers (CF) surrounded by unmyelinated fibers. The connections of mesaxon (Mx) are the extensions of the Schwann cell membrane are clearly seen. ×10,000. 3b, The Schwann cell observed on wrapping (WR) to the surrounding collagen fibers. ×15,000. 4, Ultrathin section of peripheral nerves in BT showing myelinated and unmyelinated Schwann Cells. The myelinated Schwann cells, showed attenuated Schwann cell processes arranged circumferentially forming an “Onion bulb” (OB). The cluster of unmyelinated Schwann cell can be seen with atrophied axon. The axonal cytoplasm is less electron dense than Schwann cell cytoplasm. ×7000.

thick, of the entire fascicles embedded in Spurr’s resin were examined after staining with toluidine blue (Himedia Laboratories Pvt. Ltd, Bombay). These preparations were used for a general survey and for counts of MY and UMY fibers. Photographs of the entire cross section of each fascicle along with a micrometer scale were obtained by a PM-10 ADS camera, Olympus microscope and enlarged to 1000 times. From these photographs myelinated (MY) and unmyelinated (UMY) fibers were counted and the measurement of their diameters were made by the method of Espir and Harding (10).
The frequency distribution of the UMY and external diameters of all the MY fibers in each fascicle was ascertained by separating them into groups increasing by 1 \( \mu \). The photographs of the fascicle within the perineurium area were enlarged after finding their mean radius, and the densities of the UMY and MY fibers were calculated per sq. mm of the intraperineurial area.

Ultrathin sections were cut in an ultramicrotome (MT2, Porter blum, U.S.A.) and stained with uranyl acetate and lead citrate solutions (E. Merk, India). The sections were observed under an electron microscope H-300. The accuracy of the observations by light microscopy was checked. Montages were made of electron micrographs taken at about 2000 times magnification and enlarged to about 8000 times. Measuring the respective grids-spaces by light microscopy controlled the exact final enlargement and was compared with enlarged electron micrographs. One to 4 areas, equivalent to about 5000–10,000 sq. \( \mu \) per fascicle, of 2 or 3 fascicles were studied in each case. Quantitative studies of UMY and MY fibers were made. Using the same apparatus, both UMY and MY fibers were counted. Their diameters were measured on electron micrographs after being enlarged to near 8000 times, at which magnification they could be separated reliably into groups differing by 1 millimeter. Discrimination of the frequency distribution of the diameter of UMY axon (AX) was thus possible into about 8 groups increasing by 0.2 \( \mu \). Usually, over 300 UMY AX were counted in each case. Densities of the UMY and MY were calculated as numbers per sq. mm. No correction was made for shrinkage of the tissue during preparation.

RESULTS

In Borderline Tuberculoid (BT). The semi-thin sections of the nerves revealed uniformly distributed MY fibers with few dispersed blood vessels (Fig. 1). The significant change in the diameter of the AX and MY sheath varied between BT and LL patients. Using light and electron microscopy of the entire cross sections the quantitative measurements of UMY and MY fibers in BT patients were made. The diameter of UMY fibers ranged from 0.5 \( \mu \)m to 3.8 \( \mu \)m. Their distribution ranged from 6% to 32% (Fig. 5a). The diameters of UMY fibers that were in close relation to endoneurial blood vessels were generally very small. The external diameter of MY fibers was measured by extrapolating the population of UMY fibers in the partial areas to the whole area of the fascicles. It ranged from 2 \( \mu \)m to 22 \( \mu \)m and their distribution varied from 9% to 22% (Fig. 5b). At the ultrastructural level, it was observed that the UMY fibers were extensively involved with degenerative and regenerative changes. UMY fibers manifested regeneration in the form of dense groups of very small and intact UMY AX (Fig. 2). In the regenerative AX, mesaxon connecting the AX and the basement membrane of SC was clearly seen (Fig. 3a). Proliferation of SC and prominence of endoneurial collagen fibers were also noticed. In some cases, collagen fibers increased and few SC, phagocytic in nature, were in the process of engulfing the adjacent collagen fibers (Fig. 3b). Onion-like bodies were observed, which are probably bionecrotic parts of nerves (Fig. 4). The MY group showed hypertrophy of Schwann cells with prominent nucleus and well-preserved collagen fibers. The regenerating AX were oval-shaped and some of them revealed the beginnings of MY sheath formation. However, \textit{M. leprae} organism and their debris were not observed in any of these sections (Figs. 7 and 8).

Multiple layers of basal lamellae separated by ground substance accompanied the proliferation of EC of endoneurial blood vessels. The EC and basement membrane contained many granules and were surrounded with prominent inflammatory cells. The mononuclear leukocytes were often observed forming a perivascular cuff around blood vessels in the epineurium and perineurium. Such cuffing consisted primarily of macrophages. Occasionally, circulating infected monocytes were also observed occasionally within vascular lumen. Endothelial membranes were seen with multiple infolding and often with finger like protrusion around the blood vessels. The EC were hypertrophied with enlarged nuclei, often causing narrowing of the vascular lumina. (Fig. 9). In some cases, this hypertrophy of EC was up to such an extent that the lumen of the blood vessels got completely obliterated (Fig. 10). However, the degree of obstruc-
tion of the lumen of the blood vessels due to the hypertrophy of EC varied extensively. In some cases, the lumen was completely closed and in others the lumen had narrowed, but not closed completely.

In Lepromatous Leprosy (LL). The quantitative variation of the diameter of UMY fibers in LL patients varied between 0.5 µm to 3.8 µm and their distribution in entire cross section ranged from 2% to 20% (Fig. 6a). On the other hand, the external diameter of MY fibers ranged from 2 µm to 22 µm, and their distribution varied between 3% and 25% (Fig. 6b). The ultrastructural changes were characterized by degeneration of the SC, AX, and MY sheath. *M. leprae* were present singly or in clusters. Clumping of cytoplasm, neural filaments, and neural tubules indicated degenerative changes of SC. The organisms were usually seen within the electron transparent zone (ETZ) around the bacilli in the SC, cytoplasm (Figs. 11 and 12). The endoneurial blood vessels in LL showed patent lumen lined by degenerative EC. The nucleus was small and no hypertrophy of EC was observed. The basement membrane was thin, with visible endothelial cell junction (Fig. 13). Large numbers of intact bacilli were also noticed in the EC with ETZ. In some EC, the bacilli were seen being released into the lumen through endothelial cell membrane rupture (Fig. 14).

**DISCUSSION**

The quantitative study showed the correlation between axon diameter and myelin sheath diameter, and indicated a linear correlation with the thicker sheath surrounding the larger AX. This feature is probably useful for deciding whether an AX that has no myelin sheath is truly UMY or it has become degenerated. This might be a reflection of the changes in nerve conduction velocities and axonal degeneration and regeneration or segmental demyelination in neuropathies. These observations are in agreement with earlier findings (9).

The ultrastructural study has shown significant changes in the peripheral nerves and their endoneurial blood vessels in BT and LL patients. Various workers (23, 12, 30, 20) have reported that SC are the main target cell in leprosy. Our study using electron microscopic techniques has convincingly confirmed that beside the SC, the EC of blood vessels also harbor *M. leprae* frequently.
Electron microscopic changes of peripheral nerves in BT and LL patients have indicated interesting findings in understanding the interrelationship between bacilli and the host cells. In BT nerves, the UMY SC showed degenerative and regenerative changes with severe destruction of the nerve elements.

The increases in collagen fibers suggest that the SC may play a very important role in the production of collagen in neuropathy, possibly due to lack of blood supply. The continued destruction of SC in absence of AX multiplication and excessive production of collagen fibers may be responsible for the destruction of normal nerve architecture and for the prevention of axonal regeneration. Other workers (15, 29, 30) have proposed a similar concept. Further, they have suggested that SC in these nerves give off multiple cytoplasmic processes, which form close relationship with AX and also with the collagen formation.

The reason for greater phagocytic activity of surrounding matter by SC in UMY fibers is not clearly understood. Similar phagocytic activity of SC was also noticed by earlier
workers (24, 32, 33). However in addition, the phagocytic activity by the perineurial cells was also noticed by other workers (31) who suggested that the bacilli are ingested into axoplasm by the phagocytic activity in the growth cones of the regenerating AX. Further, it was noted that regenerating AX were small in size and the mesaxons were connected with the regenerating AX, and the surface membrane of the SC. The growing tip of the regenerating AX migrate freely in the body fluid until they reach the final posi-
FIGS. 11, 12.  
11, Ultrathin sections of peripheral nerve from LL patient showing unmyelinated Schwann cell containing numerous axons and two intact bacilli (B) in Electron transparent zone. The clumping of cytoplasm and neural fibers were also seen. ×10,000. 12, Ultrathin sections of LL nerve showing unmyelinated Schwann cell with well preserved basement membrane (BM), and single intact *M. leprae* (ML). ×12,000.
Figs. 13, 14. 13, Ultrathin sections of endoneurial blood vessel in LL patients showing open lumen with small nucleus. Many *M. leprae* organisms are seen inside endothelial cell (EC) in Electron transparent zone (ETZ). The endothelial cell junctions (EJ) are also clearly noticed. ×10,000. 14, Higher magnification of endothelial cell of endoneurial blood vessels from LL patients containing many *M. leprae* organisms. The bacilli are being release from the endothelial cell into lumen of blood vessel. ×30,000.
tion in the nerve. Subsequently, SC infold the entire length of the regenerating AX behind the growing tip. It seems that in this free and naked stage of regeneration, AX engulf leprosy bacilli in the peripheral nerves. The onion-like bodies found in the BT nerve lesions resembled the plasmosome of alveolar cells. These onion-like bodies may be the remnants of the myelin (Figs. 11 and 12). These observations are in agreement with previously published findings (12, 20, 23). In BT leprosy, it was observed that almost every dermal nerve present in the localized lesion showed a presence of inflammatory cells, which destroyed large portion of nerves. The perivascular and perineurial cuffing by mononuclear cells is an indication of immune mediated inflammatory process.

Ultrastructural examination of the endoneurial blood vessels revealed that the basement membranes are multilayered, separated by ground substance indicating repeated episodes of injury to the vascular endothelium. The most interesting findings were the hypertrophy of EC noticed at various levels as observed by others (2, 4, 6, 22). Recently, (28) in an experimental study it was observed that the activation of infected EC led to thickening of the cells and narrowing of the lumen of blood vessels. In addition, these workers suggested that, since the endoneurial blood circulation is responsible for supplying the nutrients for maintaining metabolic activity of the nerves, their occlusion could be a major cause of nerve damage. However, the extent of swelling of EC in the experimental study did not lead to closure of the vessel. We hereby report a complete obstruction of the endoneurial blood vessels by hypertrophied EC which is frequently noticed in the nerves of BT patients. The ischemia caused by this, could play a major role in nerve degeneration leading to neuropathy and neural pain in these patients even after chemotherapy.

In contrast, peripheral nerves in LL cases were infected with large numbers of *M. leprae*. The number of inflammatory cells was very few when compared with that of BT cases. Further, an abundance of the organisms in SC showed foamy degeneration, followed by disintegration of AX leading to endoneurial fibrosis. The proliferation of perineurial cells, increase in endoneurial collagen, and growth of macrophage granuloma caused a pronounced thickening of the nerve. Ultrastructurally, leaving aside the SC, parasitization of other cells like EC of blood vessels and the perineurial cells was also noticed. The presence of *M. leprae* in SC in the ETZ confirmed the finding of other workers (15, 20, 15). The bacilli in various states of degenerations were seen inside phagosomes of SC. However, some SC were found loaded with intact bacilli indicating their incapability in killing *M. leprae*. Some workers (25, 26) have described a molecular mechanism of *M. leprae* gaining entry into the SC of peripheral nerves. Further, they have demonstrated that the binding of *M. leprae* to the SC is mediated by surface proteins of the bacillus binding via α-dystroglycan to the α-2 isoform of laminin found in SC. The present study has also indicated that there was no significant hypertrophy of EC and therefore the lumen of the blood vessels remain patent. However, EC contained many bacilli in the ETZ. These organisms appeared to be solid and are therefore probably viable. Further, the bacilli were found in large numbers inside the cells, indicating their intracellular multiplication. In certain situations, the rupturing of EC due to excessive bacterial growth and release of *M. leprae* in the lumen of blood vessels was clearly noticed. It is therefore obvious that the released bacilli in the blood vessels are the direct evidence for hematogenous spread of the disease. Therefore, the present study provides a proof for transmission of *M. leprae* infection in the nerve through blood stream leading to active phagocytosis of *M. leprae* by SC from the circulation due to blood nerve barrier damage (2). The present study also suggest that the small endoneurial blood vessels may play an important role for propagation of *M. leprae* infection and progression of the disease in the nerve in LL.

In conclusion, we have now shown that the mechanism of nerve damage in BT and LL forms of leprosy are different. In BT, it is probably the result of immune-mediated inflammation of the nerves damaging SC and further compounded by vascular occlusion, causing ischemia. In contrast, in LL there is infection of SC by *M. leprae* leading to their damage. There is no vascular occlusion but the EC appear to be a source of propagation of infection as the organisms actively multiply in them and are then re-
leased into the lumen of endoneurial blood vessels.

Acknowledgment. We thank Prof. V. I. Mathan, Consultant, National Institute of Epidemiology (ICMR), Chennai, and Dr. M. Mathan, Consultant, Tuberculosis Research Center (ICMR), Chennai, for their comments and constructive criticisms. We are grateful to Dr. Ashok Mukherjee, former Director of the Institute of Pathology (ICMR), New Delhi, for his critical suggestions. We would also like to thank to Mr. V. S. Yadav for statistical analysis and Mr. H. O. Agarwal and Mr. N. Dubey for photography.

REFERENCES


CASE REPORTS
Leprosy and Psoriasis: An Enigmatic Relationship¹
Sunil Dogra, Inderjeet Kaur, and Bhushan Kumar²

ABSTRACT
The relationship between leprosy and psoriasis has been controversial since ancient times. Based on the fundamental importance of nerve involvement in the pathogenesis of leprosy and psoriasis, it has been hypothesized that leprosy patients may be protected from developing psoriasis. There are only sporadic reports of coexistence of these two diseases as evidence of this negative association. We report a 64-year-old male patient with borderline leprosy and psoriasis. Recent advances in the elucidation of pathogenesis of both diseases have contributed to the understanding of this enigmatic relationship. Various genetic, immunological, and structural alterations in leprosy and psoriasis as discussed could be responsible for the rare coexistence of these two diseases in a given patient.

RESUMÉ
La relation entre la lèpre et le psoriasis est controversée depuis les temps les plus anciens. D’après l’importance fondamentale de la composante neurale dans la pathogénèse de la lèpre par rapport au psoriasis, il a été supposé que les patients hanséniens sont probablement protégés contre le développement du psoriasis. Il n’y a que quelques articles sporadiques sur la co-existence de ces deux maladies qui suggèrent une association négative. Nous rapportons ici le cas d’un patient masculin de 64 ans souffrant de lèpre borderline et de psoriasis. Les avancées récentes sur la pathogénèse de ces deux maladies ont permis de mieux comprendre cette relation énigmatique. Des altérations variées d’origine génétique, immunologique et structurelles comme discutées ici associées à la lèpre et le psoriasis pourraient être responsables de la co-existence rare de ces deux maladies chez un même patient.

RESUMEN
La relación entre lepra y soriasis ha sido controvertida desde tiempos muy antiguos. Sobre la base de la importancia de la afección a nervios en la patogénesis de la lepra y la soriasis, se ha postulado que los pacientes con lepra podrían estar protegidos contra la soriasis. Lo esporadico de los reportes sobre la coexistencia de las dos enfermedades es más bien una evidencia de esta asociación negativa. En esta comunicación informamos el caso de un paciente masculino de 64 años, con lepra BL y soriasis. Los avances recientes en la elucidación de la patogénesis de las dos enfermedades han contribuido a entender esta enigmática relación. Diversas alteraciones genéticas, inmunológicas y estructurales en la lepra y la soriasis podrían explicar la rara coexistencia de estas dos enfermedades en un paciente dado.

Controversies about the relationship between leprosy and psoriasis have existed since the time when people considered psoriasis to be a form of leprosy. The biblical term “lepra” included what is now called psoriasis (11). Undoubtedly, many psoriatic patients suffered the same physical and mental abuses as lepers of that era. This confusion between leprosy and psoriasis lasted for almost 19 centuries when it was

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realized that the two diseases are entirely different and have nothing common, not even the clinical appearance. In fact, in the latter half of the 20th century, a report on large number of leprosy patients followed up for about 40 years indicated a rarity of psoriasis among them (13). This observation stimulated the interest of many workers who have tried to explore the hypothesis that leprosy and psoriasis rarely develop in the same patient. With the better understanding of etiopathogenesis and immunological alterations in the two diseases, the basis for the rare occurrence of both leprosy and psoriasis in an individual is becoming clearer. So in a complete turn of events, the occurrence together has become a rather interesting rarity, and only a few such cases have been reported. We report another case of rare co-existence of the two diseases.

A 64-year-old male patient, presented to us with complaints of erythematous itchy scaly lesions all over the body with a history of relapses and remissions for a duration of 9 yrs. The patient was a treated case of borderline lepromatous (BL) Hansen’s Disease and had received 3 yrs of multi-drug therapy MDT-MB 13 yrs back. On examination, the patient had well defined erythematous, plaque lesions with silvery scales over the extensors of the limbs, trunk, and scalp involving almost 35% of the body surface area (Fig. 1). Nails were involved in the form of pitting, subungual hyperkeratosis, and onycholysis. Old lesions of Hansen’s disease were almost inapparent without any evidence of clinical activity. Sensory loss was present over the area of distribution of both lateral popliteal nerves and the right ulnar nerve with grade I deformity of both feet and the right hand. Plaque type lesions were randomly distributed over limbs without any particular sparing of anesthetic areas. The patient was diagnosed as having extensive psoriasis. In view of the large body surface area involvement, the patient was started on tablet methotrexate (0.5 mg/ kg/week). Patient responded well to the treatment in 6 weeks with reduction in erythema, scaling, and infiltration.

There have been only sporadic reports of this co-existence published in the literature (3, 8, 9, 12, 14). An early report from Israel stated the rarity of psoriasis among leprosy patients (13). To further explore this hypothesis, Kumar, et al. (5) carried out a questionnaire survey to be filled out by physicians at leprosy centers in different parts of the world. In this survey, out of 145,661 cases of leprosy, only 20 individuals had psoriasis. Nerve involvement is fundamental in the pathogenesis of leprosy. Mycobacterium leprae has the special characteristic of invading nerves, resulting in neuritis and nerve damage. However, in the pathogenesis of psoriasis an increasing number of biochemical and clinical studies also provide strong evidence for the functional role of cutaneous nerves and their neuropeptides. Psoriatic lesions have a significantly larger number of nerves with increased content of neuropeptides. The actions of neuropeptides like substance P (SP), vasoactive intestinal peptide (VIP), and calcitonin-gene-related peptide (CGRP) are of great significance in the inflammatory and proliferative process and symmetrical distribution of lesions in psoriasis (4, 7, 10). It has been documented that the damage to sensory nerves...
results in clearance of psoriatic lesions in anesthetic areas, and that neuropeptide-modulating drugs like capsaicin, peptide T, somatostatin, spantide, etc. have some beneficial role in psoriasis (2–7). Destruction of cutaneous nerve fibers and the consequent absence of neuropeptides from leprous skin is a well-known observation. Hence, it could be that the neuropathy caused by M. leprae infection results in structural and functional alterations in the cutaneous sensory nerves, so that the process of neurogenic inflammation, which seems to be an integral part of the psoriatic disease process, is inhibited.

Some workers have suggested that genetic factors may play a role in protecting psoriatic patients from leprosy. Population studies have found significant association of HLA-DR 2 and HLA-DQWI with leprosy, whereas in psoriasis there is high frequency of HLA–A1,–B8,–B16,–B17, CW6 and DR7 with reference to the general population. Recently most investigators have focused on the MHC class I region, with particular interest on the HLA–CW 6 allele in psoriatic patients. Now it has been established that T lymphocytes play major role in the pathogenesis of psoriasis with Th-1 type of cytokine profile triggering the chain reaction of cellular and molecular networks that culminate in the formation of a psoriatic plaque. There is increased activity of the reticuloendothelial system in patients with psoriasis in the form of enhanced metabolic, phagocytic and chemotactic functions of the polymorphonuclear leukocytes (7). This is contrary to what is observed in leprosy in which the involvement of the reticuloendothelial system and phagocytic system is characterized by reduced activity of peripheral blood mononuclear leukocytes and macrophages and depressed T-cell functional state (7).

Recently, apoptosis has been implicated to play an important role in the lymphocytic alterations in leprosy because a highly significant increase in the level of spontaneous apoptosis in leprosy patients as compared to controls has been reported (6). Apoptosis might represent a strategy of the immune system to eliminate infected cells. If apoptosis is a regular phenomenon in leprosy, in psoriasis the keratinocytes acquire an apoptotic resistant phenotype attributed to the over-expression of Bcl-X, and cell survival gene products, and other growth-regulatory or cell cycle changes (7).

In our patient, psoriatic lesions were distributed randomly over the normal as well as previously involved hypoesthetic skin areas. It is known that an adequately treated patient may have some regeneration in the affected nerves that were not completely destroyed. According to the hypothesis outlined above, the occurrence of lesions on the hypoesthetic areas can be explained.

Leprosy though is one of the oldest diseases known to mankind; several mysteries and basic facts about M. leprae and the disease they produce are still unexplained. Various genetic, immunological, and structural alterations in leprosy and psoriasis as discussed above could be responsible for the rare co-existence of two diseases in an individual patient.

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Mechanisms Involved in Peripheral Nerve Damage in Leprosy with Special Reference to Insights Obtained from In Vitro Studies and the Experimental Mouse Model

Tannaz J. Birdi and Noshir H. Antia

ABSTRACT

The histopathological observations of Khanolkar and Iyer, that M. leprae has a predilection for nerves, first highlighted the central role of peripheral nerves in the pathology of leprosy. It is now well recognized that nerve damage in leprosy will still continue to be an important problem in control and rehabilitation despite the presence of more efficient therapy. The multiplicity of mechanisms postulated, identified, and demonstrated in the last three decades has received little recognition from the scientific community at large. This review is therefore an attempt to collate these multiple studies on mechanisms of nerve damage into a cohesive analysis, which would facilitate the design of future studies. The objective of this review is to focus therefore only on studies which serve to illustrate mechanisms of nerve damage.

RESUMÉ

Les observations histopathologiques de Khanolkar et de Iyer, montrant que les M. leprae présentent une prédilection pour les nerfs, a permis de mettre en lumière le rôle central des nerfs périphériques dans la pathogénie de la lèpre. Il est maintenant bien établi que les altérations nerveuses de la lèpre vont continuer à être un problème majeur dans le contrôle et la réhabilitation, malgré la présence de thérapies de plus en plus efficaces. Le grand nombre des mécanismes postulés, identifiés et démontrés depuis les 3 dernières décennies, n’a reçu que peu de reconnaissance de la part de la communauté scientifique en général. Cette revue est donc une tentative de rassembler ces nombreuses études sur les mécanismes de l’altération nerveuse au sein d’une analyse cohérente, dont le but est d’aider à l’élaboration de futures études. Cette revue a pour mission de ne se focaliser que sur les études qui permettent d’illustrer les mécanismes d’altération nerveuse.

RESUMEN

Las observaciones histopatológicas de Khanolkar e Iyer que indicaron que M. leprae tiene una predilección por nervios, subrayaron el papel central de los nervios periféricos en la patología de la lepra. Ahora está bien reconocido que el daño a nervios en la lepra continuará siendo un importante problema para el control y la rehabilitación de la lepra, no obstante la existencia de nuevas y más eficientes formas de terapia. La multiplicidad de los mecanismos postulados, identificados, y demostrados en las últimas tres décadas ha recibido, sin embargo, poco reconocimiento por parte de la comunidad científica. Esta revisión es por lo tanto, un intento de unir, de alguna manera, estos múltiples estudios sobre los mecanismos de daño a nervios, en un análisis cohesivo que pudiera facilitar el diseño de futuros estudios. Por lo tanto, esta revisión se enfoca sólo a los estudios orientados a ilustrar los mecanismos de daño a los nervios.

1Received for publication 12 November 2002. Accepted for publication 22 September 2003.
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Anatomical and mechanical factors involved in leprous neuropathy have been reviewed extensively by Wadia (103). On the other hand, Dastur (16, 18), Palande (61), and Antia (1) concluded that raised intraneural tension was an important contributing factor, and that fibrous tunnels at the joints may further exacerbate the nerve damage which results in sites of predeliction usually being located at the entrapment site.

Maintenance of the peripheral nervous system is the result of a complex balance between the two functions of the immune system viz. assistance in maintenance of nerve physiology/ regeneration (64) and defense against infections. Leprosy serves as a unique model where, due to the insidious nature of the infecting organism, Mycobacterium leprae and its predilection for peripheral nerve Schwann cells, the two functions of the immune system co-exist at least during the initial stages.

The nerve damage following M. leprae infection of the peripheral nerve can be divided into two stages. The initial phase is common to both lepromatous and tuberculoid patients and occurs despite the absence of inflammatory cells (80, 81). In the experimental mouse model, the onset and initial progress of M. leprae-induced nerve damage remained unchanged in thymectomized irradiated mice and mice treated with anti-Thy 1.2 or Cyclosporin A, further emphasizing that the early events are not immunologically mediated (82). A recent study by Rambukkana, et al. using Rag 1-knockout mice also demonstrated that nerve damage can be initiated by M. leprae infection in the absence of an immune response (69). The studies of Brand, et al. (9) suggested that M. leprae survived better within the cooler regions of the body. Therefore, the metabolic alterations in Schwann cells and/or axon as a consequence M. leprae infection have been implicated as the major factor in this phase of nerve damage (51, 56).

In the later phase, there is an influx of mononuclear cells, which is predominantly lymphocytic in tuberculoid patients and macrophagic in the lepromatous (62).

**Initial phase of nerve damage.** The initial phase of nerve damage observed in patients across the leprosy spectrum and in a proportion of contacts is characterized by an absence of inflammatory cells (80, 81). The predominant histopathological features common in lepromatous and tuberculoid patients (78, 80) include: i) sub-perineural oedema consisting of a proteinaceous granular matrix, interspersed with small pockets of collagen; ii) axonal atrophy and secondary demyelination; iii) loss of unmyelinated fibers; iv) activation of resident macrophages and fibroblast within the endoneurial space.

In lepromatous patients, M. leprae were detected mainly in the Schwann cells of the unmyelinated fibers (80, 81). Though no acid-fast bacilli (AFB) are seen in nerves of tuberculoid patients, osmiophilic structures suggestive of bacteria are observed in the electron micrographs (2). In addition, viable M. leprae were recovered from homogenates of skin biopsies from tuberculoid patients inoculated into the mouse footpad (86).

Accompanying these degenerative changes listed above, regenerative activity was also observed across the spectrum (78), thus implying that there is a balance between regeneration and degeneration with the outcome depending on the predominating activity.

These features in the initial phase of nerve damage have also been studied in the mouse model (85). The major limitation in the mouse model has been the absence of inflammatory cells and granulomatous reactions mimicking the spectral disease of the human, as well as the inability to demonstrate bacilli in Schwann cells of the affected nerves. However, the presence of nerve abnormalities in the absence of inflammatory cells and granulomatous reactions reiterates the concept of a non-immunological mode of nerve damage in the initial stages, possibly due to aberrations in Schwann cell functions. On the other hand, the absence of bacilli in Schwann cells may merely reflect differences in the affinity or the nature of neural barriers in the human and mouse models. The Swiss White (SW) mouse is a strain in which the response of host cells to M. leprae infection parallels those observed in lepromatous patients as opposed to the C57BL/6 strain in which the response to M. leprae is similar to that observed in tuberculoid patients or normal individuals (2). The histopathological features of early nerve damage parallel the observation in patients and are similar in experimentally infected SW and C57 mice (2).
Entry of *M. leprae* into the nerve, mediated by the endoneurial endothelial cells, has been suggested by Mehta (46); Dastur, *et al.* (19, 20); Boddingues, *et al.* (1–8), and Scollard (15) who have documented the presence of *M. leprae* in the endothelial cells. Scollard (15) has proposed that no difference exists in the mechanism of initial infection across the leprosy spectrum. Bacteremia has also been reported in leprosy patients across the spectrum (12, 30). This is in keeping with the histopathological features of early nerve damage.

*M. leprae* entry into Schwann cells is the most important event in the induction of nerve damage. Based on studies utilizing murine dissociated Schwann cell cultures, early entry of *M. leprae* (within 6 hours) is observed only with viable bacteria (14). Such forced entry into macrophages has also been reported for *M. leprae* as well as (49) *Leishmania donovani* (11). Recent studies by Rambukkana, *et al.* (66) have suggested that α2 laminin and a dystroglycan are responsible for the specific predilection of *M. leprae* for Schwann cells. However, since both components are present in the basal lamina of myelinating and non-myelinating fibers, this hypothesis fails to explain the ultrastructural observations that, at least in the initial stages the *M. leprae*, are predominantly observed in Schwann cells of non-myelinating fibers (74). The studies by Choudhary, *et al.* (13) did not find evidence for a unique bacterial surface component for bacterial entry.

An important feature of infection of host cells by *M. leprae* is the down regulation cell-cell communication channels of the host cell from lepromatous patients (3, 88, 89). Once *M. leprae* have colonized the Schwann cells, expression of nerve growth factor (NGF) receptor and fibronectin secretion are down regulated (89, 90). This has been demonstrated *in vitro* in Schwann cells from SW and C57BL/6 mice. Receptor down regulation would result in decreased utilization of NGF resulting in axonal atrophy, and the decrease in fibronectin secretion would hamper regeneration. Both of these features are characteristic of the initial phase of nerve damage and are seen in patients across the spectrum (78, 80) and in the nerves of experimentally infected SW and C57BL/6 mice (5).

However, while in SW mice the damage progresses and increased demyelination is observed, in C57BL/6 the damage is arrested or delayed (18). A possible explanation is provided by the effect of *M. leprae* infection of Schwann cells *in vitro* on neural glia cell adhesion molecule (NgCAM) expression (88), Schwann cell proliferation (87), and production of collagen (89). Since none of the three parameters were affected by *M. leprae* infection of Schwann cells from C57BL/6 mice, the regenerative capacity in this strain is not hampered. In contrast, *M. leprae* infection of SW Schwann cell cultures resulted in diminished expression of NgCAM (88), which is required for efficient Schwann cell-axon interaction and is normally enhanced during regeneration. The aberrant myelination observed in infected nerves (76, 77) may be a reflection of a decrease in NgCAM expression. Schwann cell proliferation, an important prerequisite for peripheral nerve regeneration, was also decreased. In addition, SW Schwann cells secreted increased levels of collagen *in vitro* (88), which correlated with observations in sciatic nerves from experimentally infected SW (5, 85) mice and nerves of leprosy patients (78).

In summary, the initial nerve pathology observed in the sciatic nerves of SW and C57BL/6 mice infected with *M. leprae* in the footpad are similar and resemble the early changes in the nerves of lepromatous and tuberculoid patients. Therefore, it was hypothesized that the response of Schwann cells to the presence of *M. leprae* would be similar. In keeping with this hypothesis, some common aberrations were noted in the two strains in their Schwann cell response to *M. leprae* infection, which included down regulation of NGF-R expression and production of fibronectin. Nevertheless, a number of Schwann cell responses to *M. leprae* infection were also diverse in the two strains. SW Schwann cells showed decrease in proliferation, production of fibronectin, and expression of NGF-R and NgCAM, while an increase was noted in the production of collagens and laminin. Such differing responses of Schwann cells to *M. leprae* infection in the two strains in the early stages may have important implications for the later stages of nerve damage. First, it may result in differing degrees of Schwann
cell mediated regenerative activities in the two strains and this may explain the progression of early pathology to extensive damage only in SW strain (5). Secondly, such a response in patients’ nerves would probably provide signals to different populations of inflammatory cells and consequently contribute to the differing composition of the infiltrating cell population in lepromatous and tuberculoid nerves (44, 104).

Concurrent with the metabolic damage induced, the bacteria continue to multiply intracellularly within the Schwann cells. It appears that *M. leprae* growth is equally supported across the leprosy spectrum and in Schwann cells of SW and C57BL/6 mice (unpublished observations). However, the immune response generated by the host is decisive in determining the later sequence of events.

**Later phase of nerve damage.** The hallmark of the later phase of nerve damage is the presence of inflammatory cells. Studies by Dastur, *et al.* (18, 19, 20); Job, *et al.* (31, 32); and Ridley (68), among others, have attempted to elucidate the pathology of the later phase of nerve damage across the spectrum. The central question that arises is, “What is the signal for the inflammatory cell influx and how is it regulated?”

One of the earliest theories put forward was that the inflammatory response especially in tuberculoid patients was the result of autoimmunity (42). Auto-antibodies in leprosy have been reported against several nerve components (24, 105). In addition, human nerves and skin have a number of antigenic determinants in common with *M. leprae* (58, 65, 99). Many of these epitopes are heat-shock proteins (hsp) (36, 60). In animal models, it has been shown that *M. leprae*-infected macrophages attack the Schwann cells, not only in the presence, but also in the absence of detectable *M. leprae* (94), and *M. leprae* sensitized T cells also react with Schwann cell components (92). However, studies by Mshana, *et al.* (54) and Ghaswala, *et al.* (27) failed to consistently find antibodies against neural antigens. Lymphocyte proliferation in response to neural antigens was also absent (25, 55). Thus, it is suggested that autoimmunity may only contribute to the exacerbation of the lesion.

On the basis of the studies cited in the previous section, Schwann cells can be viewed as more than just supportive cells for *M. leprae* multiplication, or as mere antigen depots. Instead, the initial response of Schwann cells to *M. leprae* infection may have important and divergent influences on the immunological profile of lepromatous and tuberculoid nerves.

An alternative hypothesis is that the mononuclear cells crossing the barrier in the course of normal surveillance (38) encounter antigen presented by antigen-presenting cells (APC) leading to their activation, which in turn signals the influx of fresh cells.

Though the Schwann cell is capable of presentation of mycobacterial antigens on its cell membrane, this expression on the membrane is probably a result of integration of bacterial antigen with host membrane components during bacterial entry rather than active processing by Schwann cells (5).

Nevertheless, *M. leprae*-infected Schwann cells from both strains of mice are capable of sensitizing lymphoid cells in the murine dissociated Schwann cell culture system (50). However, this ability was dependant on the sensitization level of the lymphocytes prior to co-culture with Schwann cells, the antigen used, and the requirement of accessory cells (50). In addition, an increase in the fibroblast population in culture enabled dissociated Schwann cells to induce lymphoproliferation to *M. leprae* and 65Kd antigen even in the absence of adherent cells, demonstrating that fibroblasts could undertake the role of accessory cells, and in conjunction with infected Schwann cells, precipitate an immunological attack (50). This is important since macrophages at the site of old lesions are paralyzed/senescent.

The role of fibroblasts, as well as Schwann cells, in leprous neuropathy was also indicated in studies using immune modulated mice (82). Progressive, late nerve damage associated with demyelination was significantly reduced in mice treated with anti-thy 1.2 antibody where, along with the depletion of T-helper cells, fibroblast—which express thy 1.2 marker—would also be affected. In contrast, thymectomized and irradiated mice, where both T and B cell populations would be affected leaving the fibroblast population intact, showed similar intensity of nerve damage as the untreated.
animals. This indicates an important role for fibroblasts in lepromatous neuropathy.

The presence of MHC class I products and mycobacterial antigens on the surface of the Schwann cell (6, 95) may result in "by-stander" damage due to direct cellular cytotoxicity. Studies have demonstrated the lysis of *M. leprae*-infected Schwann cells by sensitized T cells *in vitro* (84).

In tuberculoid patients, the influx of inflammatory cells functions as a double-edged sword. The influx of macrophages enhances the regenerative process (63). In addition, the lymphocytes stimulated by *M. leprae*-antigens presented on Schwann cells are able to activate macrophages to kill intracellular *M. leprae* (50). Simultaneously with these beneficial effects, the release of protease results in collagen breakdown, which exacerbates granuloma formation (64). Reactive oxygen intermediates (ROI), produced by both Schwann cells (51) and macrophages as a consequence of the inflammatory process, results in further nerve damage at the site of the granuloma.

In lepromatous patients, the invading macrophages further down regulate the NGF-R (89), thus depriving the cell from utilizing the available NGF, leading to slow nerve damage. In addition, the NGF levels have also been reported by Facer, *et al.* (25, 26) to be decreased in lepromatous patients. Macrophages simultaneously enhance Schwann cell proliferation and NgCAM expression, thus aiding in the regenerative process (77, 88). The transforming growth factor-β (TGF-β) is important in the development and repair of the peripheral nerve (47). Conversely, it is also implicated in peripheral neuropathies, such as experimental allergic neuropathy (EAN) and experimental Wallerian degeneration (71). TGF-β has also been reported to enhance Schwann cell proliferation, up regulate NgCAM, and enhance collagen synthesis (89). These functions of TGF-β, along with its immunosuppressive action, allow us to propose that TGF-β secreted by *M. leprae*-infected macrophages from lepromatous patients/SW mice may be responsible for the decreased regenerative activity in these nerves. This is supported by the observation of Khanolkar, *et al.* (35) that maximum TGF-β was observed within lepromatous lesions.

In addition, further invasion by lymphocytes is probably curtailed by the production of suppressor factors by the infected Schwann cells (47) and the invading *M. leprae*-infected macrophages (4, 72). The data of Mehta, *et al.* (47), indicate that *M. leprae* infected murine Schwann cells, though initially stimulating lymphocytes (96), release a factor that results in cell death probably due to apoptosis. These lymphoid cells are then engulfed by Schwann cells and slowly degraded in culture (88). Apoptosis of antigen-specific T cells in lesions of experimental allergic encephalomyelitis (EAE) and EAN has been identified as an effective mechanism in stopping neural inflammation. It may be the consequence of production of a glucocorticoid by *M. leprae* infected Schwann cells (108, 109). The possibility also exists that *M. leprae* infected Schwann cells like astrocytes (99) may be secreting a tumor necrosis factor (TNF)-like factor. The refractoriness of factor production to cycloheximide treatment of *M. leprae*-infected Schwann cells (47) implicates the release of glucocorticoid resembling substances (97), which may explain the predominantly macrophage infiltration in nerve granulomas of lepromatous patients.

**Silent nerve damage.** The studies of Shetty, *et al.* (29, 73, 84) and Srinivisan and Gupte, *et al.* (93) on silent nerve damage, which is a major concern in patient management, have extensively documented that neuropathy progresses in patients after the infection is cured and even in the absence of inflammatory cells. Much less in known about the mechanism by which this occurs.

Nevertheless, there is evidence that the demyelination observed is a consequence of atrophic changes in the axonal compartment (78). It has been demonstrated that this axonal atrophy is due to hypophosphorylation of the axonal neurofilaments (87). It is of interest to note that the degenerative disorders of the central nervous system are characterized by the presence of hyperphosphorylation, whereas in leprous nerves there is decreased phosphorylation of the neurofilaments resulting in their degradation. The observation that mycobacterial antigens persists in nerves long after clinical cure (84) suggests that this persisting antigen may be important for the continued progression of nerve damage.

Preliminary findings by Shetty, *et al.* (83)
demonstrated that the decreased phosphorylation of neurofilaments was also seen in mice inoculated in the footpads with either viable or heat-killed *M. leprae*. Treatment with the TGF-β and heat shock proteins has been shown to inhibit mammalian cyclin dependent kinase (CDK) (27).

In a culture system using macrophages Chan, et al. (10) have shown that lipoarabinomannan (LAM) from *M. tuberculosis* and *M. leprae* inhibited the enzyme protein kinase C (PKC). The neuron specific kinase, responsible for phosphorylation of neurofilament proteins, CDK5 and PKC are known to share common inhibitors (43). CDK5 may therefore follow the same pattern of inhibition (40, 79). Increase in phosphatase activity, an indicator of dephosphorylation of NF, has been reported in leprous nerves and skin lesions by Dastur and Dabholkar (17). Thus, it is possible that the persisting antigens may interfere with the functions of the regulatory enzymes leading to hypophosphorylation.

Further evidence for the role of *M. leprae* antigens being involved directly in silent neuritis is obtained from the study of Croft who reported that in 32% of all impaired nerves studied, nerve function did not improve despite prednisolone therapy (15). Since prednisolone would only decrease inflammation and has no effect on the bacterial antigenic load, this further emphasizes the importance of *M. leprae* antigens in mediating silent neuritis.

**Reactions in leprosy.** The acute inflammatory response in Type I reactions is of special concern since it significantly exacerbates nerve damage. Histopathologically, the lesions show all the characteristics of a delayed-type hypersensitivity reaction (57, 68) with an influx of mainly CD4 lymphocytes, especially of the Th1 class (52, 53, 59, 102, 106, 107). There is an increase in IL-2 and TNF-α, which confirms a shift to the Th1 subtype during a reaction (34, 100, 101). Possibly due to this shift, the humoral immunity during Type I reaction seems to be diminished (100). However, a shift to Th2 may also occur since there is an increase in mRNA for IL-4 in some of the lesions (53, 102, 107).

While reactional episodes can occur at anytime during the course of the disease, recent studies suggest that their incidence has increased especially among lepromatous patients in the first year of multidrug therapy (MDT) (98). This may be due to the cidal drugs in the MDT regimen which result in the suppressor factors which are dependent on viability of *M. leprae* and are therefore no longer being produced by infected macrophages (4, 72) and Schwann cells (47), while the killed bacilli act as antigen depots, which serve to stimulate an inflammatory response. The reduced levels of TGF-β reported in reactional lesions further supports this hypothesis (15).

An alternative mechanism has been postulated by Rook, et al. (70) who suggests a neuroendocrine control of inflammation, wherein destruction of C fibers would diminish the activity of neural feedback pathways due to decreased secretion of active peptides. This in turn would prevent activation of the hypothalamus pituitary axis, resulting in local deactivation of cortisol, thereby precipitating an inflammatory response. While the first hypothesis is restricted to lepromatous patients where suppressor factors play a dominant role, destruction of C fibers, which is common to lepromatous and tuberculoid patients (78, 80) suggests that the mechanism postulated by Rook, et al. may explain reactions at both ends of the spectrum. The involvement of the Cortisol-Cortisone shuttle may also explain the clinical observations that reactional episodes are more frequent during pregnancy (23) since the enzymes of the shuttle are affected by progesterone and pregnancy (96).

While specificity of lymphoid cells isolated from nerves of non-reactional tuberculoid patients is against mycobacterial antigens and not neural antigens (22, 54), studies on antibody responses to neural antigens have resulted in conflicting results [reviewed by Desikan (21)]. The observation that sera from all patients with erythema nodosum leprosum (ENL) contain demyelinating antibodies is of particular interest (N. F. Mistry, personal communication). Nevertheless, since systemic manifestations are rare and there is no evidence of a specific reaction precipitating mycobacterial antigen (50), the difference may lie in local factors such as differential activation and tissue distribution, as well as the status of the host immune response and genetic restriction.
Acknowledgment. The critical suggestions of Drs. V. P. Shetty and N. F. Mistry in the preparation of this manuscript are deeply appreciated.

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Incomprehension

After reading the ILA Technical Forum report, many articles and editorials in specialized reviews, the latest Technical Bulletin from ILEP, the Resolution taken at the ILA Congress in Salvador (Brazil), e-mail exchanges from an anglophone discussion list (leprosy mailing list), and participating in debates at the latest International Congress on Leprosy and listening to leprosy experts and personnel « in the field », it is clear that a great difference exists in the opinion between many experts on one hand and, World Health Organization (WHO) on the other, about the analysis of the state of leprosy in the world and the measures that should be taken to control it. According to the vast majority of experts, WHO is unceasingly carrying out its leprosy « elimination » policy, apparently convinced of being enlightened while, in fact, they are taking risks without basing newly elaborated strategies on a rigorous scientific method.

The split between WHO and ILEP, as well as the absence of dialogue among all partners involved (this could have taken place, for instance, during the ILA Technical Forum or during the International Leprosy Congress), are truly regrettable.

Most observers are divided between feelings of incomprehension and indignation. It therefore appears urgent to hold a meeting with all partners involved in leprosy control to try and develop a consensus policy and broad strategies based upon rigorous criteria.

The current resurgence of certain great endemic diseases such as tuberculosis and trypanosomiasis, that were thought to be under control a few decades ago, should naturally incite us to be very cautious.

—Dr. Pierre Bobin

General Secretary,
Association of French Speaking Leprologists (ALLF)
du dernier Congrès International sur la Lèpre, sont vraiment regrettables.

La plupart des observateurs sont partagés entre incompréhension et indignation et il nous semble très urgent que l’ensemble de tous les partenaires de la lutte contre la lèpre se retrouvent autour d’une table pour essayer d’obtenir un consensus sur la politique à mener et les grandes stratégies à élaborer, en fonction de critères rigoureux.

La résurgence actuelle de certaines grandes endémies qui semblaient, il y a plusieurs dizaines d’années, bien contrôlées telles que la tuberculose ou la trypanosomiase, doit, si besoin était, nous inciter à la plus grande prudence.

On ne raye pas comme cela d’un trait de plume une maladie « millénaire », en décidant administrativement qu’elle va être très prochainement éliminée.

—Dr Pierre Bobin

Secrétaire Général
de l’Association des Léprologues de Langue Française (ALLF)

ERRATA

Due to an editing oversight in the September issue of 2003 (Vol 71.3), the title of the original article by C. K. Job, Gregory McCormick, David Scollard, and Richard Truman (p. 231) was misspelled. The correct title of the article is “Electron Microscope Appearance of Lepromatous Footpads of Nude Mice.” The editorial staff apologizes for any inconvenience this error may have caused.

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TO THE EDITOR:

Historical data indicate that Greece belongs to the group of countries with very low leprosy endemicity. Sporadic newly detected Greek cases of active disease are mainly referred by dermatologists to our unit with a prevalence rate much lower than 1:100,000 dermatologic outpatients. The disease has therefore been eliminated as a public health problem, but not completely eradicated (4). For leprosy patients and their contacts, consultation, laboratory investigation, World Health Organization (WHO) recommended multi-drug treatment (MDT), hospitalization, and follow-up are free of charge.

As has occurred in other European countries, since 1990 an influx of approximately one million migrants from Eastern Europe (mainly Albania), the Middle East, Africa, the Indian Subcontinent, and South-East Asia have entered Greece (total population ten million).

A retrospective study (1988–2000) regarding 25 Greek and five foreign newly detected (incident) leprosy patients, as well as 40 relapsed Greek cases was carried out. Relapses were old cases who, years after being discharged from the hospital and with repeatedly negative clinical and smear examinations, presented with new signs and symptoms of the disease verified by histopathologic and smear examinations (bacterial index, BI). Drug sensitivities were not assessed in the relapsed cases.

Case classification across the disease spectrum was based on clinical picture, histopathology, bacterial index from skin lesions (BI), lepromin test, and epidemiologic history. Therapeutic decisions are always based on WHO treatment recommendations (7).

Disease type distribution, yearly relative relapse rates (relapsed leprosy cases were the numerator and yearly followed up ex-leprosy patients were the denominator), and prevailing symptom at diagnosis (progressive skin lesions or a leprosy reaction) were analyzed either alone or as related to disease duration, age, gender, and residence (rural or urban).

There was no significant difference between Greek incident and relapsed cases with regard to disease type distribution (Table 1). After 1992, no more paucibacillary (PB) cases were detected. Between incident and relapsed cases, there was no difference by gender (Table 2) nor prevailing symptom that led to diagnosis. Skin lesions were a more common presenting symptom (incident cases 70%, 21/30; relapsed cases 55%, 22/40) than leprosy reactions (incident cases 30%, 9/30; relapsed cases 45%, 18/40).

When estimating disease progression in the relapsed cases, it was observed that four remained PB (10%), seven progressed from

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1 Received for publication 19 November 2002. Accepted for publication 15 October 2003.
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PB to multibacillary (MB) (17.5%), and the remaining 29 cases were MB from the moment of diagnosis (72.5%). Despite disease duration of the relapsed cases, which from the moment of first diagnosis was 29.4 ± 10.6 yrs, range 8 to 48 yrs, there was no difference in disease type distribution within incident cases at first diagnosis. Relapse rate and disease duration did not differ between men and women. The number of ex-leprosy patients followed up annually series presented a declining trend and a negative compound growth rate (exponential trend, \( Y = 395.7 (0.92)^t \), \( t \)-statistic –5.96, \( p < 0.001 \), compound growth rate –7.6%) (Table 2).

Regarding the age of Greek active leprosy cases, there was a significant difference between incident PB (N = 5, mean 26.6, range 6 to 56 yrs) compared with incident MB (N = 20, 55.9, range 33 to 79 yrs, \( p < 0.001 \)), but not between incident MB and the relapsed cases (N = 40, 64.05, range 42 to 90 yrs, Bonferroni \( p \)-values, one way ANOVA). Only 2 children younger than 14 yrs were detected in the whole study period (diagnosed in 1989).

Comparisons regarding possible associations of active case detection with residence (rural or urban) revealed that rural residence (villages) was five times more frequent in incident cases (19 of 25, 76%, “exact” CI 54.9 to 90.6) when compared to relapsed cases, who are mainly living in cities, mostly in the Athens greater area and throughout the country (25 of 40, 62.5%, “exact” CI 45.8 to 77.3, \( \chi^2 \), Yates corrected, \( p = 0.005 \), odds ratio (OR) 5.3, “exact” CI for OR 1.5 to 19.4). In contrast, there was no association of patient residence with gender (incident, relapsed, and overall).

Foreign patients represented a small proportion of total active leprosy cases (N = 5, total N = 70, 7.1%, “exact” CI 2.4 to 15.9), coming from endemic countries (Table 1). After 1992, only one imported case (LL) was detected.

**DISCUSSION**

In Greece during the second half of the 20th century, rapid socioeconomic development, rapid urbanization, substantial improvement of living standards, nuclear family pattern predominance, and a lack of differentials in the access to health care services were gradually achieved. These factors are closely associated with a disease decline, reduced exposure and transmission even in endemic areas, irrespective of leprosy control interventions (1). Thereafter, secondary prevention, a good dapsone
monotherapy program in conjunction with yearly clinical and BI examinations aiming
a permanent smear negativity of registered cases and the significant BCG coverage of
the general population have further contributed to the epidemiologic pattern of a
dying out disease where the few cases that occur have a predominance of lepromatous
leprosy (1, 5).

A constant policy of our center is that all current and former household contacts of new
patients should be invited for examination. As a rule, nuclear family members do present
at least once for clinical, BI and histopathologic examinations, as the only cost effec-
tive method of active case finding (1).

The majority of new cases and especially those seen after 1992 seem to represent a
“hidden prevalence” which was not perceived before (6). A clear evidence for this is
documented by more frequent rural residence of incident cases, the lack of difference
in age between incident MB and relapsed cases, as well as by the homoge-
nous disease type distribution in both patient categories at first diagnosis.

In our elderly ex-leprosy patients, stigma is based on memories of compulsory segre-
gation, isolation in leprosaria, and discriminatory lows in the past (2, 3). These condi-
tions resulted in concealment which, in association with socioeconomic develop-
ments, might explain why the majority of relapsed cases lives in urban areas.

The number of yearly examined, prominently elderly, ex-leprosy patients is not
constant and gradually declines as a function of mortality, life conditions influencing
compliance of elderly people in general, and stigma-related fatigue. According to
previous regimens, all smear negative MB cases first treated with dapsone monother-
apy had to remain under lifelong treatment with dapsone. Re-treatment with MDT of
all these old cases requires intense health education and is gradually obtained.

—Kyriakos Kyriakis, M.D.
Dermatologist in Charge
West Attica General Hospital and
Leprosy Center
Athens, Greece

REFERENCES


Dr. Paul W. Brand, one of the greatest physicians of all time, well known to all leprologists of his generation, passed away on 8th of July, 2003, at the age of 89. He was a pioneering surgeon, an excellent teacher, a compassionate leprologist, and a dedicated missionary.

He was born in South India in July, 1914 of British parents, studied medicine in the University College Hospital, London, and later qualified for the FRCS diploma. His father Jessi Brand died of malaria while serving as a missionary to the tribal people in Kolli Hills, situated in the mountain ranges of South India. His mother took over the work of her husband and served the tribal folk until her death at the ripe old age of 96. Service to the suffering, underprivileged, and the poor was his inheritance.

The call to serve as a medical missionary in India came to him in 1946 from Dr. Robert G. Cochrane, the then principal of the Christian Medical College, Vellore, who was desperately searching for a surgeon to satisfy the university requirements for recognition of the MBBS degree offered by the college. Dr. Brand was a born teacher. His graphic descriptions of disease presentations were brilliant and were long remembered by many of his students; I was fortunate to be one of them. His deep concern and care for patients touched and moved each one of his students to whom he was a role model.

It seems to me that it was God’s plan that brought together the world famous leprologist, Dr. Robert Cochrane and Dr. Brand, the young enthusiastic, ingenious and compassionate surgeon. In 1947 when, at the leprosy hospital at Chinglepet, Dr. Cochrane showed him the useless, deformed and disabled hands of leprosy patients that no surgeon had ever cared to touch and to repair, Dr. Brand saw the challenge before him. He started his careful research into the pathology and pathogenesis of deformities in leprosy and into methods to reconstruct their paralyzed hands and feet. His pioneering work in the correction of deformities caused by leprosy changed the lives of thousands of grateful patients. He also trained many surgeons from different parts of the world using the facilities at Schieffelin Leprosy Research and Training Center, Karigiri, and at Christian Medical College, Vellore.

Another area of research he was engaged in was the pathogenesis of plantar ulcers. He established that the main course of these ulcers was loss of sensation due to nerve damage and not just leprosy. This finding led to the adoption of measures to heal, to protect, and to prevent the damage to insensitive hands and feet. Specialized sandals, made out of microcellular rubber, were found suitable to prevent the formation of plantar ulcers and to stop their recurrence. A rubber mill to manufacture microcellular rubber was established at Schieffelin Leprosy Research and Training Center, Karigiri because it was not profitable for any commercial rubber company to manufacture this product.
Dr. Brand was the first one to demonstrate that nerve damage was localized to subcutaneously placed nerves and to suggest that *M. leprae* multiplied at the cooler regions of the body. This finding led to the path breaking experimental studies by Charles Shepard who succeeded in growing *M. leprae* in the footpads of mice.

At various periods during his tenure in Christian Medical College, Vellore, Dr. Brand held the posts of Professor of Orthopedic surgery, Associate Director and Principal. He was also consultant surgeon at Schieffelin Leprosy Research Center at Karigiri. He established the New Life Center, Vellore, as a model rehabilitation center for leprosy patients. This Center simulated a village environment and was located at the residential area of the Christian Medical College campus, in an effort to dispel the stigma that was so prevalent even among medical professionals. Correcting deformities to restore the self-respect of patients and to integrate them into society was his cherished goal.

In 1966, after 19 years of service in India he moved to the U.S.A. on invitation to take up the position of Chief of Rehabilitation Branch at the National Hansen’s Disease Center at Carville. He worked there for 20 years and established a well-equipped and well-staffed research unit to study the complications of insensitive hands and feet, their prevention and management. His methods for prevention and management of plantar ulcers are now extensively used for treatment of patients with diabetes mellitus who have similar problems. His contribution to the understanding of pain is monumental. He emphasized the role of pain, which protects and preserves and is a blessing. When he retired in 1986 from the U.S. Public Health Service, he moved to Seattle and continued his teaching work as emeritus professor of Orthopedics in the University of Washington.

During his career, Dr. Brand received many awards and honors. He was awarded the Hunterian professorship of the Royal College of Surgeons in 1952, and the Albert Laskar award in 1960. Queen Elizabeth honored him with a title of the Commander of the Order of the British Empire in 1961. He served as President of the Leprosy Mission International based in London and was on the Panel of Experts on leprosy of the World Health Organization. He was one of the main architects of the All Africa Leprosy Rehabilitation and Training Center in Addis Ababa, Ethiopia, and the Schieffelin Leprosy Research and Training Center at Karigiri, India. A biography was written on him entitled, “Ten fingers for God,” by Dorothy Clarke Wilson. He authored several books that were best sellers among Christian literature. They are “Fearfully and Wonderfully made,” 1981, “In His Image,” 1984, and “Gift of Pain,” 1993. He also wrote a standard reference book for hand surgeons entitled “Clinical Mechanics of the Hand.”

Dr. Paul W. Brand, with all the honors he received and with all his greatness, remained a simple and humble Christian leprosy worker. He was a man of integrity and witnessed for his convictions forcefully and effectively with gentleness and respect. He exemplified in his life that excellence in medicine was not just knowledge and skills but the application of them to serve and to give one’s best to the cause of the poor, needy and the neglected. His many contributions to the care of leprosy patients will be long remembered.

He leaves behind his loving wife, Dr./Mrs. Margaret Brand, six children and twelve grandchildren. Mrs. Brand is also a leprologist who has received international acclaim in the study and management of eye complications in leprosy. We, his students, friends, colleagues and patients, share with the family the sadness and the loss. Nonetheless, we celebrate his great contributions and the privilege of having known him, having had him as a teacher, friend, colleague, and caregiver. We thank and praise God for his wonderful and blessed life that has been a blessing to many.

—Dr. C. K. Job
My first meeting with Paul Brand was at the Proctor House Mission in Bombay when I had gone to enquire of him the danger of contracting leprosy during surgery. He assured me that this was a mere myth as he and his staff had kept a record of needle pricks during surgery for over a decade with no untoward consequences.

We next met when I advertised for a physiotherapist for our hospital resulting in an interview visit by Furness, Brand’s senior physiotherapist and the husband of Brand’s secretary (also an old leprosy patient). This resulted in obtaining his brother-in-law, Walter Jennings, as our physiotherapist, who served our hospital for almost two decades, a former leprosy patient who had both his hands operated on by Brand. The excellence of Brand’s surgery not only permitted him to undertake excellent physiotherapy, but also to keep typed records of patients and their surgeries. As a result of our discussions, he eventually performed “many tail” operations on paralyzed hands with excellent results, for he could judge the tensions of the graft better than most surgeons.

Another outcome of Furness’ visit was the visit of Ernest Fritschi to Pune in order to observe our plastic surgery approach to deformities of the face in this disease, undertaken under primitive conditions with patients as the only assistants.

This led to several decades of interaction between Dr. Brand, myself, and his colleagues in Vellore and Karigiri. Paul visited Kondhwa and observed Sir Harold Gillies undertaking surgery during his visit in 1958.

My first visit to Karigiri was in 1959, to attend the First International WHO conference on Rehabilitation on Leprosy Deformities, where I was familiarized with the care of the ulcerated foot in leprosy. At this meeting, Ernest Price also demonstrated his interesting observations on the footprints of leprosy patients. This meeting provided due recognition to the pioneering work of Brand in this field. It also led to support from the U.S. Department of Health, Education, and Welfare for several of our leprosy and burn activities including surgery, research, and rehabilitation.

This was followed by a series of exchanges between our institutions and my personal interaction, not only with Paul and Margaret, but also with the Karats, Selvapandian and Anthony Samy at Katpadi—a fruitful exchange.

During a six week visit to our department at the J. J. Hospital in Bombay, Dr. Robert Cochrane provided interesting information as to how he had inveigled a new orthopedic surgeon of Vellore into undertaking the correction of deformities of patients sent to him from the Victoria Hospital in Chingleput. Thanks to Paul, Vellore became a mecca of surgery for leprosy. Many of the surgeons from various countries of the world while visiting Vellore also visited us in Bombay and Kondhwa.

The contributions of Cochrane and Brand will always remain as landmarks in leprosy with Vellore as their “home.”

It is heartening for me to see that leprosy is one of the few diseases of poverty that has shown a definite decrease. This is also reflected in the decrease of its deformities, even though eradication will remain a distant dream till necessary poverty is banished by concerted political action at all levels and in all countries.

—Dr. N. H. Antia
International Journal of Leprosy

Dr. Gopal Ramu

1924–2003

Dr. Gopal Ramu, the well known leprologist from India, passed away on 25 July 2003, at the age of 79.

Dr. Ramu was born in Kerala and had his early medical education at Indore. His initial career started in the state of Holkar. Even while working in general medicine, he showed a keen interest in leprosy. He pursued his leprosy interest both in the field and in the clinic when he started working at Rewa (M.P.). It was at this time that he was involved in research on lathyrism under the Indian Council of Medical Research. His association with Dr. C. G. S. Iyer during the lathyrism research enabled him to look for a better place to pursue his interest in leprosy, and to join the Central Leprosy Teaching and Research Institute, Chengalpattu.

Dr. Ramu was greatly instrumental in building up the clinical research at two major leprosy institutions in India, the Central Leprosy Teaching and Research Institute (CLTRI), Chengalpattu (Tamilnadu) from 1962–1976, and the Central JALMA Institute for Leprosy Research (CJIL), Agra from 1976–1986. Besides these two major institutions, Dr. Ramu made significant contributions to leprosy research in his earlier days at Rewa and in his post-retirement days at Kumbakonam and Coimbatore (Tamilnadu).

Dr. Ramu was an outstanding expert on all aspects of clinical leprosy and blended his keen observational instincts with good science. Together with the veteran leprologist Dr. K. Ramanujam and with the support of Dr. C. G. S. Iyer and Dr. K. V. Desikan, he studied several aspects of the clinical manifestations of leprosy and its complications. Thus, the 1960’s saw some important contributions in leprosy from him through this group. He was also instrumental in developing a rational method for scoring of clinical lesions in leprosy which greatly facilitated the follow-up of patients during clinical trials.

When Dr. Ramu moved to CJIL at Agra in 1986, he saw an opportunity not only to build good clinical services but also to exploit important research opportunities that had become available by then through developments in the serology of leprosy. This work very much complimented his earlier interest in lepromin reaction among patients and contacts.

Of his several accomplishments, one of the most important was his ability to motivate young workers who came in contact with him. The environment and encouragement provided by him resulted in several individuals joining in leprosy research. Wherever he was, he set a good example of diligence and perseverance.

As an individual, Dr. Ramu was greatly liked by everyone who interacted with him. He was most helpful and generous, both to his associates and patients. Deeply interested in music and religion, he spent the last part of his days with his daughter at Coimbatore (Tamilnadu) continuing to provide advice on leprosy research.

The 1960’s saw a great deal of resurgence in leprosy research in India and Dr. Ramu came onto the scene at the right time and made a great impact in better understanding the disease and its management. His memory will be cherished for a long time both by his fellow workers and patients.

—Dr. S. K. Nordeen
**Notice.** Several extra copies of the old issues of *The International Journal of Leprosy* are available from the business office. Due to a shortage of storage space, some of these must be discarded soon. If you wish to obtain any of these back issues of the JOURNAL, please contact Dr. Paul Saunder-son by e-mail: psaunderson@leprosy.org.

**Notice.** *The International Journal of Leprosy* is now available on-line by visiting our website at http://www.leprosy-ila.org. This provides the most convenient access to the JOURNAL on-line. You can also renew your membership, or join if you are not already a member of the ILA. The JOURNAL will accept submissions electronically, as well.

**Academic Meeting at Kalyan.** The Indian Association of Leprologists—Maharashtra Branch in collaboration with Bombay Leprosy Project organized a seminar on “Leprosy—from a Practicing Dermatologists Point of View” on Sunday 22.06.2003 at Kalyan.

This seminar was organized for the members of the “Kalyan-Dombivli Dermatologists Club,” a newly formed local academic association.

Issues such as the Role of standard Prednisolone in management of reactions, Role of newer drugs like Cyclosporin, Pentoxyphylline and other useful drugs like Thalidomide availability to needy patients in managing chronic/recurrent reactions, as an alternative line of treatment were discussed at length. Dr. R. Ganapati and Dr. V. V. Pai were the Resource Persons.

Discussion on methods of recording the past treatment details of patients in a “Treatment Graph” experimented and prepared by BLP was also demonstrated. The objective of such innovative exercise was a scientific study of the treatment details given to patients either referred or institute cases, helpful in deciding a rationale management.

Clinically interesting cases (staying in Kalyan area) with recurrent type II reaction put on Thalidomide were also demonstrated and discussed. The seminar was sponsored by M/s Jansen’s Cilag Pharmaceuticals Ltd.

The Editorial office received the following letter from the Leonard Wood Memorial.

TO: Friends of the Leonard Wood Memorial
Subject: New Scientific Director

I am most happy to inform you that effective September 1, 2003, Dr. Robert Gelber will officially join the Leonard Wood Memorial as its new Scientific Director.

As you may know, Dr. Gelber has been working in leprosy, both as a clinician and researcher, particularly in the field of chemotherapy, for almost four decades. During this time, he has published well over 100 articles and written major chapters in prestigious textbooks. He comes to us from his position of Clinical Professor at the University of California, San Francisco and also Senior attending physician of the TB control program at San Francisco General Hospital.

We are delighted to have hired someone who is highly qualified, both in the field of leprosy and tuberculosis. He is excited and enthusiastic about this position and we look forward to a rich and rewarding association with Dr. Gelber.

Please join us in welcoming him.

Sincerely,

August Zinsser III
President
LWM Board of Trustees
ILA GLOBAL PROJECT ON THE HISTORY OF LEPROSY

ACADEMIC NETWORK MINUTES OF INAUGURAL MEETING,
SORIA MORIA CONFERENCE CENTER, OSLO
Friday, 5 September 2003

Present: Jo Robertson (chair), Jaime Benchimol, Harriet Deacon, Deborah Emmitt, Mark Harrison, George Joseph, Sanjiv Kakar, Simonne Horwitz, Anwei Law, Laurinda Maciel, John Manton, Renisa Mawani, Yara Monteiro, Chandi Nanda, Diana Obregón, Shubha Pandya, Biswamoy Pati, David Scollard, Magali Romero Sá

Apologies: Bernardino Fantini

1. THE PROJECT AND ITS INTERESTS

Jo Robertson summarized the Project’s activities to date. The last funding period of twenty months ended in May 2003, and the Project has now entered a bridging stage of funding, provided by the Sasakawa Foundation, until future funding of a further three years is assured.

The main aims of the Project are, firstly, to build an online database of archives on leprosy, held in numerous locations throughout the world, and secondly, to establish and maintain a network of researchers who are working on different aspects of the history of leprosy. This network is expected to be self-perpetuating, that is, the members will maintain contact amongst themselves once the Project has made them aware of each other’s existence through its website and activities.

**Oral History**

Anwei Law explained the oral history component, which will begin once further funding is assured. Oral history “makes history more rounded,” as it is related by the people actually involved. When making oral histories, it is important to include families, and both younger and older generations as this establishes continuity. Guidelines on making oral histories will be developed, and other expressions produced by people who have been affected by leprosy will be identified, such as poetry, artwork and music. The idea is to develop a network that is dedicated to making oral history, as there are not enough resources for Anwei Law and her team to carry out all the recording themselves. She pointed out that anyone over the age of seventy is a “fragile resource,” so their identification and oral history will be a priority.

2. INDIVIDUAL RESEARCH AREAS

This item on the agenda was postponed, but Jo Robertson pointed out that many members’ research interests are described on the Academic Network page of the Project website.

3. RESEARCH TOPICS OF INTEREST FROM THE POINT OF VIEW OF PEOPLE IN THE FIELD

David Scollard, as Editor of the *International Journal of Leprosy and Other Mycobacterial Diseases*, talked about the submission of articles by members of the network. The journal is a bio-science publication, but has always accepted the occasional article of historical interest. There is a commitment to support the history of leprosy, and submitted papers from social science disciplines will be reviewed by appropriate social scientists. Readers of the journal, mainly leprosy doctors and researchers around the world, must see the articles as useful. David Scollard invited members of the leprosy history network to submit articles of historic interest and to ask themselves how their submission is useful in the field, how it could help medical workers, scientists, and others.

4. RESEARCH OPPORTUNITIES

Mark Harrison explained how he intends to make the history of leprosy one of the main areas of research at the Wellcome Unit, Oxford, and will invite applications for this. He will be putting in research proposals to the Grants Committee—at least one large one, or maybe two smaller ones. In addition, individual research applications can also be made to the Wellcome Trust, from academics from the European Economic area. Wellcome collaborative grants make available a relatively small amount of money to develop specific research projects, in order to facilitate travel between the
two units for meetings and conferences, as well as the employment of researchers. Mark Harrison invited suggestions for collaborative projects. Biswamoy Pati asked whether Ph.D. students could apply, Mark replied that there was nothing in the rubric against it, and that all the basic details concerning this can be found on the Wellcome Unit website.

5. STRATEGIES FOR THE FUTURE OF THE NETWORK

Jo Robertson outlined three main strategies. Firstly, an electronic discussion forum will be set up, the importance of which was made clear by the recent pre-conference exchange of emails, mainly concerning use of terminology in historical articles. Secondly, suggestions on papers to be submitted to history of medicine conferences are welcome. Thirdly, possible collaborations among members of the network, which Jo Robertson left open for discussion.

Discussion

Diana Obregon—The electronic forum could be used to share bibliographic information as each academic is not necessarily aware of other publications in the field.

Sanjiv Kakar—It will also be useful for sharing information on conferences worldwide.

George Joseph—The submission of papers to conferences is normally more productive when in panels, rather than sending individual papers, which are often difficult for organizers to place in the program. He is in the early stages of planning a conference for late April or early May in the United States. The American Association for the History of Medicine (AAHM) will meet in Birmingham, Alabama, during the first week of May and in conjunction with the leprosy conference most likely to be held in New Orleans, Louisiana, it may be possible to arrange a visit to Carville at the same time. George Joseph suggested the circulation of the papers prior to the conference to allow a more advanced level of discussion.

John Manton—There is a problem with studying leprosy history in “tropical” Africa (i.e., Africa except South Africa). It would therefore be useful to underpin networks of Africa scholars and/or have a symposium in an African University. It would be difficult to get funding but it is important to do so.

Jo Robertson—If anyone knows of any other academics in the leprosy history field, let her know and she will establish communication with them.

Henk Menke—It may be useful to define one main historical problem as a group, and see how the research of each country relates to it.

Jo Robertson—This approach is important, and could be fulfilled by the research projects outlined by Mark Harrison. Also, on the academic network web page, there is information on members’ publications and research interests. Academics need the freedom to go in the direction that their work leads them, and would prefer not to be pinned down to one particular research area.

George Joseph—Maybe three or four areas could be established to begin with but one would be too constraining.

Jo Robertson—We could look at the current areas being researched by the network and compile a list of core issues.

Jaime Benchimol—Agreed that it is too early to identify specific research areas. What would be valuable is an appraisal of what has been done in the field, e.g., leprosy and public health.

Sanjiv Kakar—Oral history is one dominant theme that we already have.

Jo Robertson—The politics of oral history are being handled carefully in the proposal for further funding, as there were problems with this area previously. We are trying to incorporate it once more.

Harriet Deacon—Oral history is a methodology, not an analytical approach. By pointing out that it is part of every history may be a convincing argument in its favor.

Anwei Law—The problem arises from not seeing oral history as a good source of history due to a lack of understanding of its use in different contexts.

Harriet Deacon—Asked whether the Project has to clear all methodologies with WHO.

Jo Robertson—Now that the Project is strong, we have received an email from WHO that is virtually contractual, stating that the website content must be cleared by WHO. This issue will go to the Steering Group to be debated. Jo Robertson listed the members of the Steering Group, as not
all the network members were aware of whom it comprised. There is now a page on the website with this information.

**Chandi Nanda**—Asked whether it is possible to identify some people who have been cured of leprosy, who can be brought into the network.

**Jo Robertson**—This is an academic network. Anwei Law will develop a network of people to gather oral histories.

**Henk Menke**—The main group working for leprosy patients to date has been doctors and nurses. The historical method is relatively new. We need to make our work clear to those in the field, not only through publications but also meetings. If it is limited to historians, we may miss the important goal.

**David Scollard**—The last ILA Congress in Salvador, Brazil, is an example of how historians and present day medical workers have already come together. The history symposia during this Congress were very successful. There are people from all fields at this regular, international conference, so to have history also represented is very good. Hopefully we can find future ways in congresses to put forward particular historical problems and issues.

**Jo Robertson**—It was a very international gathering, and people who had had the disease were also responding.

**Harriet Deacon**—One of the dangers of classing us as a leprosy network limits the focus to that disease. However, it is useful to make comparisons with issues surrounding syphilis, AIDS and other diseases, to see how policies that develop around these matters relate to leprosy.

**Jaime Benchimol**—Emphasis also needs to be given to national studies; tuberculosis is an important comparison.

**Anwei Law**—This kind of study should not be limited to diseases, but human rights issues too.

**Jo Robertson**—Bernardino Fantini is developing a human rights program and wants to include leprosy.

**David Scollard**—Expressed a desire to publish the abstracts of the current conference in the *International Journal of Leprosy*. Members were asked to make any final adjustments to their abstracts, and send them to Jo Robertson as soon as possible.

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**INDEPENDENT EVALUATION OF THE GLOBAL ALLIANCE FOR THE ELIMINATION OF LEPROSY (GAEL)**

**June 13, 2003**

Released by WHO 4 July 2003, the evaluation of the Global Alliance for the Elimination of Leprosy, GAEL, was drafted by an independent panel of six, led by Dr. Richard Skolnik. With the exception of Professor Michel Lechat, the evaluators are not part of the leprosy community. They based the evaluation on a review of literature, communications, documents and approximately 100 widely representational interviews.

Richard Skolnik (Team Leader), Florent Agueh, Judith Justice, Michel Lechat.
The George Washington University, The University of Louvain, and The University of California at San Francisco.

**ABSTRACT**

This is an independent evaluation of the Global Alliance for the Elimination of Leprosy. It assesses the extent to which the Alliance has contributed to the goal of eliminating leprosy as a public health problem. This evaluation was based on a review of literature, documents, communications, and almost 100 informant interviews.

The evaluation team believes that the Alliance has added important value to the goal of eliminating leprosy as a public health problem. It has mobilized political commitment, financial resources, and free drugs. It has helped to improve the management and reach of multi-drug therapy. It has energized a number of leprosy programs. During the course of the Alliance, 16 of 22 endemic countries have been deemed to have met the goal of elimination.

In addition, at the country level, the Alliance appears to be functioning well. Most countries are actively leading and coordinating their leprosy programs. Collaboration is good, with the World Health Organization (WHO) playing an advisory role and non-government organizations (NGOs) in-
involved in a range of leprosy efforts in conjunction with WHO and government.

Despite these important successes, the Alliance is not adding the value that it could add and this poses threats to country leprosy programs and to the reputations of collaborators on leprosy work. Relations among some collaborators at the global level are very bad. Concerned NGOs, physicians, and scientists have raised important questions to WHO about technical, operational, and strategic matters but they have not been resolved. In addition, some collaborators do not have a clear understanding of the aims of the Alliance, or a clear agreement on how the Alliance should be governed. There are also strong views among some collaborators that the Alliance is too embedded in WHO and that WHO has not been sufficiently consultative in its management of the Alliance.

This is already mid-2003, and the target date for elimination that was set by the World Health Assembly and extended by the Alliance is very close. There will continue to be significant numbers of leprosy patients after the goal of elimination has been achieved. In addition, there will also be needs at the global level for advocacy efforts, funds for leprosy activities, and exchanges of information and best practices among those working on leprosy. At the local level, all countries will need to lead their leprosy programs in sound ways. If these measures are not addressed effectively, some of the important gains on leprosy will be lost.

For these reasons, the panel believes that the Alliance must be rebuilt and refined immediately. Much of the global work of the Alliance would be convened and lead by the NGO and foundation movement. These activities would focus on ensuring effective advocacy, as needed, and promoting learning and input into country programs on technical, operational, and strategic issues. They would build on earlier work by the International Association of Anti-Leprosy Associations (ILEP), the International Leprosy Association (ILA), and the Sasakawa Memorial Health Foundation. They would include all who work with leprosy, including the private sector and groups of people affected by the disease.

If not already doing so, countries should organize their leadership around a country-level leprosy task force. WHO should play the advisory role to country programs, with effective use of input from other collaborators. The WHO should also convene a group of technical advisors, selected with the advice of others involved in leprosy, to carry out independent monitoring and evaluation of leprosy activities. The Technical Advisory Group (TAG) of WHO would have its membership strengthened, again with the advice of others.

It is also hoped that the Novartis Corporation, working with the Novartis Foundation for Sustainable Development, would continue to provide drugs and that the Sasakawa Memorial Health Foundation and the Nippon Foundation would continue to support technical cooperation and research, including through its important financial assistance.

The above approach would carry on from the work done effectively to date and would build on the comparative advantages of different actors engaged in leprosy efforts. It would also build on the unique role and commitment in leprosy work of NGOs. It would have clear and accountable roles for all actors and would be inclusive. It would also have to be based on open, transparent, and collegial relations, the lack of which would preclude any alliance from effectively supporting the important work on leprosy that will remain, even after 2005. Finally, these arrangements would help provide a sound transition to further leprosy control and rehabilitation efforts.

Obtained directly from the WHO web-site, http://www.who.int/lep/GlobalAlliance/evaluation.doc, at which the full report may be examined.

Notice. On 13–15 October 2003 a Workshop was held in Amsterdam on Leprosy Transmission and Diagnosis. During this workshop it was decided to co-ordinate research activities in this field.

A consortium supported by the WHO/TDR Special Program therefore now issues a call for interest for partners to engage in this comprehensive research program to apply modern developments in the molecular typing of M. leprae and specific
antigen/epitope definition to field studies towards better understanding of the epidemiology and transmission of leprosy, and the improved diagnosis of leprosy infection. The purpose of this call is to recruit partners to participate in working groups on:

- Assays for molecular epidemiology
- Immunology-based diagnostic assays
- Field studies related to transmission and diagnosis

The purpose of the working groups is to (i) raise funds to advance the necessary basic and operational research; and (ii) set policies, proposals, protocols, under the umbrella of the consortium.

If you are interested in participating, please submit a letter of interest to the Interim Steering Committee of the consortium briefly stating the extent of your interest in these areas, your experience and your association with leprosy field studies. A standard form and more information is available at: http://www.kit.nl/biomedical_research/htm/leprosy_research_consortium.asp

Dr. Linda Oskam Ph.D. (secretary of the Interim Steering Committee)
Research Co-ordinator Mycobacteriology
KIT (Royal Tropical Institute) Biomedical Research
Meibergdreef 39, 1105 AZ Amsterdam, The Netherlands
http://www.kit.nl/biomedical_research/

Calendar of Meetings and Events

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<td>ILEP Working Session and General Assembly</td>
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<td>19–22</td>
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<td>Dr. S.K. Noordeen</td>
<td><a href="mailto:vinodkoomar@rediffmail.com">vinodkoomar@rediffmail.com</a></td>
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AMERICAN SOCIETY FOR MICROBIOLOGY
DIVISION U SYMPOSIUM
Washington Convention Center
Washington D.C.
18–22 May 2003

The 103rd General Meeting of the American Society for Microbiology was held at the Washington Convention Center in Washington D.C., U.S.A., on May 18–22, 2003. At this meeting, Division U (Mycobacteriology) sponsored a symposium entitled, “Advances in Leprosy Research 2003 and Beyond: Following in Shepard’s FootPads.” This symposium featured a distinguished list of speakers covering a range of topics which emphasized the rapid gains in knowledge since the sequencing of the Mycobacterium leprae genome. This symposium also featured the prestigious Division U lecture, which is awarded each year to a member of Division U whose outstanding achievements have contributed to advances in mycobacteriology.

The conveners of the symposium were Drs. James L. Krahenbuhl and Diana L. Williams, both of the National Hansen’s Disease Programs Laboratory, Baton Rouge, LA, U.S.A. After a brief introduction by Dr. Krahenbuhl, the symposium commenced with this year’s Division U lecturer, Dr. Warwick J. Britton from the University of Sydney, Sydney, Australia, who presented an overview of his research on leprosy and tuberculosis vaccines. His lecture was followed by a presentation by Dr.
Williams on her work in defining a partial *M. leprae* transcriptome. Dr. Patrick J. Brennan, Colorado State University, Fort Collins, Colorado, U.S.A., presented his work on the genes encoding *M. leprae* cell wall components. Last, Dr. Thomas P. Gillis, National Hansen’s Disease Programs Laboratory, discussed his research on the use of bioinformatics to search for potential vaccine and skin test antigen candidates.

This informative and timely symposium was attended by over 350 meeting participants and each presentation stimulated thoughtful discussion. In an effort to share the proceedings of this symposium with the readership of the *International Journal of Leprosy* who were unable to attend the meeting, the four lecturers have here provided an overview of their presentations.

—Linda Adams

Chair, Division U (Mycobacteria)
American Society for Microbiology

**ABSTRACTS**

**Britton, W. J.** On the vaccine trail: from Leprosy to Tuberculosis.

The control of leprosy has improved markedly since the introduction of multi-drug therapy with dramatic falls in the prevalence of leprosy patients receiving antimicrobial therapy during the 1990’s. Despite this widespread implementation of MDT, the incidence of leprosy as measured by case detection rate has not yet fallen in major endemic countries. This justifies continuing research to understand the transmission, host response in protective immunity against *M. leprae*. Immunization to improve host response against *M. leprae* infection will also be an important component in the long term control of leprosy.

**Current leprosy vaccines.** The anti-tuberculosis vaccine, *Mycobacterium bovis* bacille Calmette-guerin (BCG) has partial efficacy against clinical leprosy. In four randomized clinical trials, BCG stimulated a degree of vaccine efficacy (VE) ranging from 34% in India to 80% in Uganda. The prospective vaccine study in Malawi demonstrated a 52% VE for BCG, with a further 50% reduction in clinical leprosy following repeat BCG immunization (1). In addition, 9 case control studies have shown that past BCG immunization, as indicated by the presence of BCG a scar, was associated with approximately 50% reduction in clinical leprosy (range 20 to 81%). The impact of widespread BCG implementation on leprosy control is difficult to quantitate, however, it is probable that BCG is one factor which has contributed to the decline in leprosy in some countries. The recent South India vaccine trial provided further evidence of the effectiveness of vaccines against clinical leprosy (2). In this study the addition of heat-killed *M. leprae* to BCG resulted in improvement in the vaccine efficacy from 34 to 61%. This is contrast to earlier studies in Malawi and Venezuela, which showed no benefit from the addition of heat-killed *M. leprae* to BCG (1). An additional finding was that the cultivatable bacillus ICRC, probably a member of the *M. avium-intracellulare* family, also confered significant protection (VE, 65%) when given as a dead mycobacterium. This provides further evidence that immunization with heterologous mycobacteria protects against *M. leprae*.

**Subunit vaccines against leprosy.** The potential of subunit vaccines against leprosy was raised by the early studies of Gelber and Brennan, which showed that crude *M. leprae* cell wall fractions and native cell wall-derived proteins protected against *M. leprae* footpad infection in mice. More recently, Ngamying and colleagues showed that *M. leprae* cytosol and membrane fractions protected against mouse footpad infection, but the cell wall skeleton of mycolyl-AG peptidoglycan from *M. leprae* was not protective (3). Therefore, protein components are essential for effective subunit vaccines. The trail to determine which *M. leprae* protein antigens induce effective immunity dates back to studies with monoclonal antibody-defined proteins in the 1980’s. These included the *M. leprae* GroEs, GroEl and 70 kDa heat shock proteins, widely shared with other mycobacteria, and the *M. leprae* 18 kD protein, which contains *M. leprae*-specific T cell epitopes but has a homologue in *M. avium*. Subsequently, native proteins were purified from *M. leprae*, including the cytoplasmic 10 kDa GroEs protein and the membrane-associated proteins MMPI and MMPII. Fractions of *M. leprae* containing GroEs
and MMPI stimulated some protective effect in the mouse footpad model. More recently, a bioinformatics approach has been employed to define M. leprae homologues of M. tuberculosis antigens known to induce protective immunity against tuberculosis, such as the secreted proteins, Antigen 85B and ESAT-6.

Our own group has focused on the M. leprae 35 kDa (MMPI) as a subunit vaccine against leprosy. This protein was first recognized by M. leprae-specific monoclonal antibodies to conformational determinants on the protein, which are also the dominant epitopes for human leprosy sera. We cloned the gene for the M. leprae 35 kDa protein (10) and found it had no homologues in M. tuberculosis or BCG, but one was present in M. avium with 94% amino acid identity (5). Recombinant 35 kDa protein expressed in the rapidly growing M. smegmatis forms highly immunogenic multimers of >900 kDa. This protein is recognized across the leprosy spectrum, so that paucibacillary leprosy patients and contacts of leprosy patients develop a strong T cell response with low levels of antibody and multi-bacillary patients demonstrate a strong antibody response and weak T cell response (4). Further, the protein elicits delayed type hypersensitivity (DTH) in M. leprae sensitized guinea pigs.

DNA vaccines expressing the M. leprae or M. avium 35 kDa proteins induced protective immunity against M. leprae and M. avium infection in mice, which was equivalent to BCG in both cases (6,7). This was accompanied by strong specific Interferon (IFN)-γ T cell responses, as well as high titre antibody responses to the conformational determinant on the protein. This establishes that immunization with a single antigen can be effective against experimental leprosy infection. Major antigens shared with M. tuberculosis and BCG may also induce protection when used as a subunit vaccines. For example, immunization with the M. tuberculosis antigen 85B as a DNA vaccine induced heterologous protection against M. leprae footpad infection in Swiss albino mice. (8).

**Improving subunit vaccines against mycobacterial infections.** Although subunit vaccines against leprosy infection show an equivalent protection to BCG in a mouse model, single protein or DNA vaccines against tuberculosis infection have generally been less effective than BCG in mice (9). Therefore, we and others have been examining ways of increasing the protective efficacy of subunit vaccines against mycobacterial infections. A number of cytokines were compared as adjuvants for DNA immunization and plasmid IL-12 was the most effective. Co-immunization with DNA-85B and plasmid IL-12 resulted in a rise in IFN-γ and T cell responses, a fall in specific antibody responses with increased protection against M. tuberculosis infection (10). Co-immunization with DNA-35 and plasmid IL-12 produced significantly greater protection against virulent M. avium infection than BCG (11). IL-12 was more effective than IL-18, another Th1-promoting cytokine, at improving DNA vaccine efficacy against mycobacterial infections (12).

We have also examined the interactions of subunit vaccines and BCG against M. tuberculosis infection. Priming with a DNA vaccine expressing the M. tuberculosis antigen 85B, followed by boosting with BCG, significantly improved the effective efficacy against M. tuberculosis to a level greater than that achieved with BCG alone (10). This may be due to focusing of the immune response against a dominant secreted antigen of M. tuberculosis. These findings demonstrate that protection against mycobacterial infections in experimental models is not limited to that achieved with BCG alone.

**Challenges for new anti-leprosy vaccines.** Although considerable progress has been made, there remain uncertainties in the understanding the complex host immunological response to mycobacteria and this influences the development of more effective anti-leprosy and anti-tuberculosis vaccines. First, the factors which determine the immunological dominance of antigens are still not resolved. Factors which may affect this include the quantity of mycobacterial protein and the timing of exposure. For example, the GroES protein is the major cytoplasmic protein in armadillo-derived M. leprae, and is a dominant antigen in host response to M. leprae (13). Secreted proteins, including antigen A85 complex, may be the first antigens encountered and appear to stimulate protective immunity against a number of mycobacteria (8,10). There may
be intrinsic properties to certain proteins, such as the multimeric form of the 35 kDa \textit{M. leprae} protein, which contribute to their persistence in the phago-lysosome and so their antigenicity.

Second, the importance of species specificity in determining the protective efficacy of individual proteins is unclear. In fact, species-specific proteins may not be the optimal vaccine candidates, although they have obvious importance as a diagnostic reagents. Recent studies by Black and colleagues in Malawi (14) have demonstrated apparent cross-reactivity between the major antigens of \textit{M. leprae}, including the 35kDa and 18kDa proteins, and environmental mycobacterial species isolated in Malawi. These antigens may stimulate pre-existing T cell responses in infected subjects in endemic regions and this may blunt the apparent effectiveness of mycobacterial vaccines in that environment.

Third, the relative contribution of different T cell subsets to protective immunity may vary between different species of mycobacteria. Both CD4 and CD8 T cells appear to contribute to effective immunity against \textit{M. tuberculosis}, although the exact role of CD8 T cells inducing protection against \textit{M. leprae} infection has not been established.

Fourth, understanding the factors controlling of T cell memory, particularly in CD4 T cells is incomplete and this has major implications for subunit vaccines against mycobacterial infections. Although protein and DNA subunit vaccines can stimulate short term protective immunity against tuberculosis and leprosy in animal models, their ability to stimulate long term protection is yet to be determined. These subunit vaccines may prove most useful in boosting immunity established by BCG or other visible vaccines.

The second major challenge for new anti-leprosy vaccines are limitations of the models for testing protective efficacy. The dynamic range of the mouse footpad infection model is low, and it is difficult to measure increased effective efficacy above that achieved with BCG. The lack of immunological reagents and complexibility of the armadillo model mean that it is not currently applicable to testing vaccines. Further, the length of time for testing individual vaccines, currently 9 to 12 months, restricts the rate of progress. In addition, it is important to confirm that the vaccine antigens induce protective immunity in mice with differing genetic backgrounds to confirm their applicability in human populations.

The third and most important challenge for introduction of new anti-leprosy vaccines is the capacity to test these in endemic regions. Will it be possible to conduct another major human leprosy vaccine trial on the scale of the recent Indian vaccine study? This would require sufficient number of new cases and the extensive infrastructure required for such a study. Another approach would be to include leprosy in future tuberculosis vaccine trials. A number of candidate TB vaccines are moving into Phase I and Phase II clinical trials. If these are to be used in leprosy endemic countries, it is be important that they have an effective anti-leprosy component, either because the antigens are shared between \textit{M. tuberculosis} and \textit{M. leprae}, or \textit{M. leprae}-related components are added to the vaccine. This will require the design and conduct of the vaccine trials to measure the effects on leprosy as well as tuberculosis in leprosy-endemic regions.

Acknowledgement. Laboratory research has been funded by the National Health and Medical Research Council of Australia, the World Health Organization, the Co-operative Research Center for Vaccine Technology and the New South Wales Department of Health. I thank E. Martin, J. Triccas, U. Palendira and A. Kamath in Sydney and Dr. P. Roche, Anadanaban Leprosy Hospital, Kathmandu, Nepal, for their collaboration and discussions. I am grateful to Dr. J. Velemer, Leprosy Mission International for his analysis of the effectiveness of BCG vaccines.

REFERENCES


functions. These data have provided us with the first insight into the transcriptome of *M. leprae* and further demonstrated the homogeneity of this species. It is anticipated that this analysis will help to identify a larger set of functional genes in *M. leprae* which will potentially help us to understand the minimal requirements for growth and replication of this pathogen. This information may lead to the identification of new drug targets, skin test antigens, and to identify factors that allow this pathogen to evade the immune system and destroy peripheral nerves.


Sequencing of the *Mycobacterium leprae* genome by S.T. Cole, *et al.* (http://www.nature.com/nature/v409/n6823/fig_tab/4091007a_F1.html) was a momentous event, comparable to the introduction of MDT (multiple drug therapy) in the 1980’s. Initial analysis indicated a genome size of about 3.3 megabases, a G/C content of 57.8%, only 1604 protein genes, 116 pseudogenes, and hence a protein coding capacity of 49.5% (these latter figures are to be compared to a size of about 4.4 megabases for the *Mycobacterium tuberculosis* H37Rv genome, a G/C content of 65.6%, 3959 protein genes, only about 6 pseudogenes, and thus a protein coding capacity of 90.8%). (These data are being constantly revised in light of more recent and ongoing analysis of bacterial genomes.) Thus, the *M. leprae* genome has undergone reductive evolution, becoming trapped and crippled. In light of such a paucity of protein coding genes, it is worthwhile to examine the cell wall of *M. leprae* from both the perspectives of known biochemical information and *in silico* analysis. This was the purpose of this review.

For instance, we have known from the early chemical analysis of the cell wall peptidoglycan of *M. leprae* by P. Draper, *et al.*, that this essential component of all eubacteria is intact and comparable to that of *M. tuberculosis* and other bacteria. Indeed, *M. leprae* apparently retains the full *mur* operon (*ftsZ, fisQ, murC, murG, ftsW, murD, murX, murF, murE*) and other related genes (*e.g.*, *murA, murB, ponA1, ponA2*). Polyprenyl-phosphates are the membrane carrier lipids for many aspects of cell wall synthesis, such as arabinose, arabinogalactan, peptidoglycan, and, as expected, *M. leprae* contains the majority of genes encoding enzymes of the non-mevalonate pathway for polyprenyl-phosphate synthesis (*e.g.*, *dxsI* and *ispC–G*). It is devoid of the *dxsII* of *M. tuberculosis*, which helped in deciding that *dxsI* is the functional gene for deoxyxylulose-5-phosphate synthase. The entire array of genes required for rhamnose synthesis (*rmlB, rmlC, rmlD, rmlA*) are present in the *M. leprae* genome, as expected, since rhamnose is a component of the key diglycosyl-phosphoryl unit joining the mycolyl-arabinogalactan complex to peptidoglycan. Most of the known genes responsible for the synthesis of mannose, the PIMs (phosphatidylinositol mannosides), LM (lipomannan), and LAM (lipoarabinomannan), are present in *M. leprae*, such as *pmmA*, the Rv3256c homolog, *pmi, manB*, the Rv2609c homolog, *pimA*, Rv2611c homolog, and *pgsA*. However *pimB* is apparently missing, which requires new thinking on the mechanism of synthesis of PIMs/LM/LAM; we do know from chemical analysis that all three types of products are present in *M. leprae*. Likewise, most of the genes for mycolic acid synthesis, modification, and deposition are present in the *M. leprae* genome, such as *fasI, fabD, acpM, kasA, kasB, accD6, mabA, inhA, umaA2,mmaA4,mmaA1,fbpA,fbpB,fbpC*, and *fbpC2*. However, as noted initially by Cole, *et al.*, *umaA1,mmaA3, and mmaA2* are missing as whole genes, and this absence is exactly in accord with the absence of methoxymycolates in *M. leprae* as reported by several workers in the 1980’s. The *pks* (polyketide synthase)-like genes of *Mycobacterium tuberculosis* responsible for the synthesis of the phthiocerol, phenolphthiocerol, and methyl branched fatty acids of DIM (dimyccerosyl phthiocerol) and the phenolic glycolipids (PGL) are receiving considerable current attention, since these products have been implicated in disease processes. In the case of *M. tuberculosis*, it has been demonstrated that disruption of the *pks10* and *pks7* genes which are clustered with *pks8,*
Similarly, the pks12 gene has also been implicated in synthesis of DIM. However, a careful analysis of the M. leprae genome indicates that pks10, pks7, and pks12 are pseudogenes; and pks 8, 17, 9, and 11 are absent.

An analysis of the intact pks genes of M. leprae from a different perspective, i.e., from the perspective of identification of the genes responsible for the synthesis of PGL-I, yields interesting information. The entire array of genes attributed to the synthesis of phthiocerol (ppsA–E) is located between ML2357 and ML2353. At a different location (ML0139) is the mas gene responsible for mycocerosic acid synthesis. The pks1 and pks15 of M. tuberculosis genes are fused as one gene in the M. bovis genome, which has been demonstrated to be involved in the synthesis of phenolphthiocerol, most likely a precursor of the M. bovis specific PGL. The M. leprae genome also contains the fused gene in concordance with the elaboration of its phenolic glycolipids. Elongation occurs with the ppsA–E cluster. Associated with ppsA–E are the genes fadD26 and drrA–C implicated in the attachment of mycocerosic acid and phthiocerol followed by transport through the membrane. Putative glycosyltransferases and methyltransferases possibly involved in the synthesis of the trisaccharide segment of PGL-I can be located in one cluster (ML0125-ML0130).

We have previously commented on the “cell wall gene cluster” of M. leprae characterized by the presence of embA–C implicated in arabinan synthesis, the fbp genes responsible for mycolic acid deposition, and glf and glfT involved in D-galactofuranose and D-galactan synthesis. A detailed comparison of gene arrangements in this cluster in the M. leprae and M. tuberculosis genomes demonstrates the absence of several genes (Rv1500 to Rv1526) from the M. leprae genome, one of which is a putative glycosyltransferase (Rv3786c). Additionally, a dedicated analysis of putative glycosyltransferases over the entire genome of M. leprae versus M. tuberculosis shows that several orthologs (e.g., Rv1212c, Rv0539, and Rv2957) representative of the glycosyltransferase families 1 and 2 are missing from M. leprae. Likewise, a cluster of at least 8 glycosyltransferases in the Rv1500 to Rv1526 region of the M. tuberculosis genome is missing from M. leprae. This evidence supports the chemical evidence for “stunted” or “truncated” versions of some polysaccharides, such as LAM, in M. leprae.

Thus, an analysis of the genome of M. leprae versus those of M. tuberculosis, M. bovis, and other Mycobacterium spp. supports the chemical analytical evidence of an intact but minimal cell wall in M. leprae.

Some relevant publications:


Gillis, T. The Use of Bioinformatics in Leprosy Research.

Bioinformatics encompasses all aspects of biological information acquisition and analysis, and combines the tools of computer science and biology with the aim of understanding biological significance. The combination of enhanced sequencing and computing power has allowed for unprecedented advances in assimilating huge amounts of raw data and the initiation of meaningful molecular modeling. Bioinformatic approaches are particularly attractive for aiding studies of M. leprae since many conventional biological tools are unavailable to investigators working with nonculturable agents.

Three major areas of bioinformatics (genomics, proteomics, and transcriptional profiling) have had and should continue to have an impact on our understanding of M. leprae and the disease it causes. Genomics has provided a working genetic blueprint for M. leprae allowing for comparisons with other mycobacterial genomes to assess M. leprae’s basic physiological capabilities, potential virulence factors and establish
molecular markers for drug resistance and strain variation. Newly developed strain identification markers using simple repeated DNA sequences should provide tools to investigate transmission patterns of leprosy and may help define risk factors involved in reinfection versus relapse of disease. Algorithms for predicting open reading frames and for categorizing location and function of *M. leprae* proteins have initiated basic studies on secreted proteins as well as proteins involved in nerve invasion and *M. leprae*-specific proteins potentially useful for detecting exposure to the leprosy bacillus through skin testing or serological testing.

Proteomic studies of the leprosy bacillus have been hampered by low protein yields from purified bacilli derived from infected animals, but have established a baseline profile of highly expressed proteins from *M. leprae*. Combining transcription profiling of the leprosy bacillus with proteomic studies may provide new insights into proteins previously lost during purification from infected host tissues and provide new antigens useful for studying immune responses during infection. In addition, newly discovered proteins can be tested for their ability to induce protective immunity and may constitute a new group of proteins with vaccine or diagnostic potential. Transcriptional profiling may allow investigators to study *M. leprae*’s gene expression at different stages of growth as well. For example, gene expression in growth-permissive cells, such as the macrophage and Schwann cell could differ and, therefore, may reveal aspects of *M. leprae*’s unique tissue tropism.

Functional genomics is the integration of predictive bioinformatics with validation through experimental biological analysis. This part of the equation remains a major challenge to workers in the leprosy field. Surrogate genetics to study *M. leprae* genes in cultivable mycobacteria as well as new approaches for “knocking in” genes to *M. leprae* are areas in need of research and development. Both approaches will benefit from bioinformatics and should continue to further our understanding of the leprosy bacillus in particular, and the host-parasite relationship in general.
**Notice.** Several extra copies of the old issues of *The International Journal of Leprosy* are available from the business office. Due to a shortage of storage space, some of these must be discarded soon. If you wish to obtain any of these back issues of the JOURNAL, please contact Dr. Paul Saunder-son by e-mail: psaunderson@leprosy.org.

**Notice.** *The International Journal of Leprosy* is now available on-line by visiting our website at http://www.leprosy-ila.org/. This provides the most convenient access to the JOURNAL on-line. You can also renew your membership, or join if you are not already a member of the ILA. The JOURNAL will accept submissions electronically, as well.

**Academic Meeting at Kalyan.** The Indian Association of Leprologists—Maharashtra Branch in collaboration with Bombay Leprosy Project organized a seminar on “Leprosy—from a Practicing Dermatologists Point of View” on Sunday 22.06.2003 at Kalyan.

This seminar was organized for the members of the “Kalyan-Dombivli Dermatologists Club,” a newly formed local academic association.

Issues such as the Role of standard Prednisolone in management of reactions, Role of newer drugs like Cyclosporin, Pentoxiphylline and other useful drugs like Thalidomide availability to needy patients in managing chronic/recurrent reactions, as an alternative line of treatment were discussed at length. Dr. R. Ganapati and Dr. V. V. Pai were the Resource Persons.

Discussion on methods of recording the past treatment details of patients in a “Treatment Graph” experimented and prepared by BLP was also demonstrated. The objective of such innovative exercise was a scientific study of the treatment details given to patients either referred or institute cases, helpful in deciding a rationale management.

Clinically interesting cases (staying in Kalyan area) with recurrent type II reaction put on Thalidomide were also demonstrated and discussed. The seminar was sponsored by M/s Jansen’s Cilag Pharmaceuticals Ltd.

The Editorial office received the following letter from the Leonard Wood Memorial.

TO: Friends of the Leonard Wood Memorial

Subject: New Scientific Director

I am most happy to inform you that effective September 1, 2003, Dr. Robert Gelber will officially join the Leonard Wood Memorial as its new Scientific Director.

As you may know, Dr. Gelber has been working in leprosy, both as a clinician and researcher, particularly in the field of chemotherapy, for almost four decades. During this time, he has published well over 100 articles and written major chapters in prestigious textbooks. He comes to us from his position of Clinical Professor at the University of California, San Francisco and also Senior attending physician of the TB control program at San Francisco General Hospital.

We are delighted to have hired someone who is highly qualified, both in the field of leprosy and tuberculosis. He is excited and enthusiastic about this position and we look forward to a rich and rewarding association with Dr. Gelber.

Please join us in welcoming him.

Sincerely,

August Zinsser III

President

LWM Board of Trustees
ILA GLOBAL PROJECT ON THE
HISTORY OF LEPROSY
ACADEMIC NETWORK MINUTES
OF INAUGURAL MEETING,
SORIA MORIA CONFERENCE
CENTER, OSLO
Friday, 5 September 2003

Present: Jo Robertson (chair), Jaime Ben-
chimol, Harriet Deacon, Deborah Emmitt,
Mark Harrison, George Joseph, Sanjiv Kakar,
Simonne Horwitz, Anwei Law, Laurinda Ma-
ciel, John Manton, Renisa Mawani, Yara
Monteiro, Chandi Nanda, Diana Obregón,
Shubha Pandya, Biswamoy Pati, David Scol-
lard, Magali Romero Sá

Apologies: Bernardino Fantini

1. THE PROJECT AND ITS INTERESTS

Jo Robertson summarized the Project’s
activities to date. The last funding period of
twenty months ended in May 2003, and the
Project has now entered a bridging stage of
funding, provided by the Sasakawa Foun-
dation, until future funding of a further
three years is assured.

The main aims of the Project are, firstly,
to build an online database of archives on
leprosy, held in numerous locations
throughout the world, and secondly, to es-
tablish and maintain a network of re-
searchers who are working on different as-
pects of the history of leprosy. This network
is expected to be self-perpetuating, that is,
the members will maintain contact amongst
themselves once the Project has made them
aware of each other’s existence through its
website and activities.

Oral History

Anwei Law explained the oral history
component, which will begin once further
funding is assured. Oral history “makes his-
tory more rounded,” as it is related by the
people actually involved. When making
oral histories, it is important to include
families, and both younger and older gener-
ations as this establishes continuity. Guide-
lines on making oral histories will be devel-
oped, and other expressions produced by
people who have been affected by leprosy
will be identified, such as poetry, artwork
and music. The idea is to develop a network
that is dedicated to making oral history, as
there are not enough resources for Anwei
Law and her team to carry out all the
recording themselves. She pointed out that
anyone over the age of seventy is a “fragile
resource,” so their identification and oral
history will be a priority.

2. INDIVIDUAL RESEARCH AREAS

This item on the agenda was postponed,
but Jo Robertson pointed out that many
members’ research interests are described
on the Academic Network page of the Proj-
ect website.

3. RESEARCH TOPICS OF INTEREST
FROM THE POINT OF VIEW OF
PEOPLE IN THE FIELD

David Scollard, as Editor of the Interna-
tional Journal of Leprosy and Other My-
cobacterial Diseases, talked about the
submission of articles by members of the
network. The journal is a bio-science pub-
lication, but has always accepted the occa-
sional article of historical interest. There is
a commitment to support the history of
leprosy, and submitted papers from social
science disciplines will be reviewed by
appropriate social scientists. Readers of the
journal, mainly leprosy doctors and re-
searchers around the world, must see the ar-
ticles as useful. David Scollard invited
members of the leprosy history network to
submit articles of historic interest and to ask
themselves how their submission is useful
in the field, how it could help medical
workers, scientists, and others.

4. RESEARCH OPPORTUNITIES

Mark Harrison explained how he intends
to make the history of leprosy one of the
main areas of research at the Wellcome
Unit, Oxford, and will invite applications
for this. He will be putting in research pro-
posals to the Grants Committee—at least
one large one, or maybe two smaller ones.
In addition, individual research applications
can also be made to the Wellcome Trust,
from academics from the European Eco-
nomic area. Wellcome collaborative grants
make available a relatively small amount of
money to develop specific research proj-
ects, in order to facilitate travel between the
two units for meetings and conferences, as well as the employment of researchers. Mark Harrison invited suggestions for collaborative projects. Biswamoy Pati asked whether Ph.D. students could apply, Mark replied that there was nothing in the rubric against it, and that all the basic details concerning this can be found on the Wellcome Unit website.

5. STRATEGIES FOR THE FUTURE OF THE NETWORK

Jo Robertson outlined three main strategies. Firstly, an electronic discussion forum will be set up, the importance of which was made clear by the recent pre-conference exchange of emails, mainly concerning use of terminology in historical articles. Secondly, suggestions on papers to be submitted to history of medicine conferences are welcome. Thirdly, possible collaborations among members of the network, which Jo Robertson left open for discussion.

Discussion

*Diana Obregon*—The electronic forum could be used to share bibliographic information as each academic is not necessarily aware of other publications in the field.

*Sanjiv Kakar*—It will also be useful for sharing information on conferences worldwide.

*George Joseph*—The submission of papers to conferences is normally more productive when in panels, rather than sending individual papers, which are often difficult for organizers to place in the program. He is in the early stages of planning a conference for late April or early May in the United States. The American Association for the History of Medicine (AAHM) will meet in Birmingham, Alabama, during the first week of May and in conjunction with the leprosy conference most likely to be held in New Orleans, Louisiana, it may be possible to arrange a visit to Carville at the same time. George Joseph suggested the circulation of the papers prior to the conference to allow a more advanced level of discussion.

*John Manton*—There is a problem with studying leprosy history in “tropical” Africa (i.e., Africa except South Africa). It would therefore be useful to underpin networks of Africa scholars and/or have a symposium in an African University. It would be difficult to get funding but it is important to do so.

*Jo Robertson*—If anyone knows of any other academics in the leprosy history field, let her know and she will establish communication with them.

*Henk Menke*—It may be useful to define one main historical problem as a group, and see how the research of each country relates to it.

*Jo Robertson*—This approach is important, and could be fulfilled by the research projects outlined by Mark Harrison. Also, on the academic network web page, there is information on members’ publications and research interests. Academics need the freedom to go in the direction that their work leads them, and would prefer not to be pinned down to one particular research area.

*George Joseph*—Maybe three or four areas could be established to begin with but one would be too constraining.

*Jo Robertson*—We could look at the current areas being researched by the network and compile a list of core issues.

*Jaime Benchimol*—Agreed that it is too early to identify specific research areas. What would be valuable is an appraisal of what has been done in the field, e.g., leprosy and public health.

*Sanjiv Kakar*—Oral history is one dominant theme that we already have.

*Jo Robertson*—The politics of oral history are being handled carefully in the proposal for further funding, as there were problems with this area previously. We are trying to incorporate it once more.

*Harriet Deacon*—Oral history is a methodology, not an analytical approach. By pointing out that it is part of every history may be a convincing argument in its favor.

*Anwei Law*—The problem arises from not seeing oral history as a good source of history due to a lack of understanding of its use in different contexts.

*Harriet Deacon*—Asked whether the Project has to clear all methodologies with WHO.

*Jo Robertson*—Now that the Project is strong, we have received an email from WHO that is virtually contractual, stating that the website content must be cleared by WHO. This issue will go to the Steering Group to be debated. Jo Robertson listed the members of the Steering Group, as not
all the network members were aware of whom it comprised. There is now a page on the website with this information.

**Chandi Nanda**—Asked whether it is possible to identify some people who have been cured of leprosy, who can be brought in to the network.

**Jo Robertson**—This is an academic network. Anwei Law will develop a network of people to gather oral histories.

**Henk Menke**—The main group working for leprosy patients to date has been doctors and nurses. The historical method is relatively new. We need to make our work clear to those in the field, not only through publications but also meetings. If it is limited to historians, we may miss the important goal.

**David Scollard**—The last ILA Congress in Salvador, Brazil, is an example of how historians and present day medical workers have already come together. The history symposia during this Congress were very successful. There are people from all fields at this regular, international conference, so to have history also represented is very good. Hopefully we can find future ways in congresses to put forward particular historical problems and issues.

**Jo Robertson**—It was a very international gathering, and people who had the disease were also responding.

**Harriet Deacon**—One of the dangers of classing us as a leprosy network limits the focus to that disease. However, it is useful to make comparisons with issues surrounding syphilis, AIDS and other diseases, to see how policies that develop around these matters relate to leprosy.

**Jaime Benchimol**—Emphasis also needs to be given to national studies; tuberculosis is an important comparison.

**Anwei Law**—This kind of study should not be limited to diseases, but human rights issues too.

**Jo Robertson**—Bernardino Fantini is developing a human rights program and wants to include leprosy.

**David Scollard**—Expressed a desire to publish the abstracts of the current conference in the *International Journal of Leprosy*. Members were asked to make any final adjustments to their abstracts, and send them to Jo Robertson as soon as possible.

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**INDEPENDENT EVALUATION OF THE GLOBAL ALLIANCE FOR THE ELIMINATION OF LEPROSY (GAEL) June 13, 2003**

Released by WHO 4 July 2003, the evaluation of the Global Alliance for the Elimination of Leprosy, GAEL, was drafted by an independent panel of six, led by Dr. Richard Skolnik. With the exception of Professor Michel Lechat, the evaluators are not part of the leprosy community. They based the evaluation on a review of literature, communications, documents and approximately 100 widely representational interviews.

Richard Skolnik (Team Leader), Florent Agueh, Judith Justice, Michel Lechat.
The George Washington University, The University of Louvain, and The University of California at San Francisco.

**ABSTRACT**

This is an independent evaluation of the Global Alliance for the Elimination of Leprosy. It assesses the extent to which the Alliance has contributed to the goal of eliminating leprosy as a public health problem. This evaluation was based on a review of literature, documents, communications, and almost 100 informant interviews.

The evaluation team believes that the Alliance has added important value to the goal of eliminating leprosy as a public health problem. It has mobilized political commitment, financial resources, and free drugs. It has helped to improve the management and reach of multi-drug therapy. It has energized a number of leprosy programs. During the course of the Alliance, 16 of 22 endemic countries have been deemed to have met the goal of elimination.

In addition, at the country level, the Alliance appears to be functioning well. Most countries are actively leading and coordinating their leprosy programs. Collaboration is good, with the World Health Organization (WHO) playing an advisory role and non-government organizations (NGOs) in-
volved in a range of leprosy efforts in conjunction with WHO and government. Despite these important successes, the Alliance is not adding the value that it could add and this poses threats to country leprosy programs and to the reputations of collaborators on leprosy work. Relations among some collaborators at the global level are very bad. Concerned NGOs, physicians, and scientists have raised important questions to WHO about technical, operational, and strategic matters but they have not been resolved. In addition, some collaborators do not have a clear understanding of the aims of the Alliance, or a clear agreement on how the Alliance should be governed. There are also strong views among some collaborators that the Alliance is too embedded in WHO and that WHO has not been sufficiently consultative in its management of the Alliance.

This is already mid-2003, and the target date for elimination that was set by the World Health Assembly and extended by the Alliance is very close. There will continue to be significant numbers of leprosy patients after the goal of elimination has been achieved. In addition, there will also be needs at the global level for advocacy efforts, funds for leprosy activities, and exchanges of information and best practices among those working on leprosy. At the local level, all countries will need to lead their leprosy programs in sound ways. If these measures are not addressed effectively, some of the important gains on leprosy will be lost.

For these reasons, the panel believes that the Alliance must be rebuilt and refined immediately. Much of the global work of the Alliance would be convened and lead by the NGO and foundation movement. These activities would focus on ensuring effective advocacy, as needed, and promoting learning and input into country programs on technical, operational, and strategic issues. They would build on earlier work by the International Association of Anti-Leprosy Associations (ILEP), the International Leprosy Association (ILA), and the Sasakawa Memorial Health Foundation. They would include all who work with leprosy, including the private sector and groups of people affected by the disease.

If not already doing so, countries should organize their leadership around a country-level leprosy task force. WHO should play the advisory role to country programs, with effective use of input from other collaborators. The WHO should also convene a group of technical advisors, selected with the advice of others involved in leprosy, to carry out independent monitoring and evaluation of leprosy activities. The Technical Advisory Group (TAG) of WHO would have its membership strengthened, again with the advice of others.

It is also hoped that the Novartis Corporation, working with the Novartis Foundation for Sustainable Development, would continue to provide drugs and that the Sasakawa Memorial Health Foundation and the Nippon Foundation would continue to support technical cooperation and research, including through its important financial assistance.

The above approach would carry on from the work done effectively to date and would build on the comparative advantages of different actors engaged in leprosy efforts. It would also build on the unique role and commitment in leprosy work of NGOs. It would have clear and accountable roles for all actors and would be inclusive. It would also have to be based on open, transparent, and collegial relations, the lack of which would preclude any alliance from effectively supporting the important work on leprosy that will remain, even after 2005. Finally, these arrangements would help provide a sound transition to further leprosy control and rehabilitation efforts.

Obtained directly from the WHO web-site, http://www.who.int/lep/GlobalAlliance/evaluation.doc, at which the full report may be examined.

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Notice. On 13–15 October 2003 a Workshop was held in Amsterdam on Leprosy Transmission and Diagnosis. During this workshop it was decided to co-ordinate research activities in this field.

A consortium supported by the WHO/TDR Special Program therefore now issues a call for interest for partners to engage in this comprehensive research program to apply modern developments in the molecular typing of M. leprae and specific
antigen/epitope definition to field studies towards better understanding of the epidemiology and transmission of leprosy, and the improved diagnosis of leprosy infection. The purpose of this call is to recruit partners to participate in working groups on:

- Assays for molecular epidemiology
- Immunology-based diagnostic assays
- Field studies related to transmission and diagnosis

The purpose of the working groups is to (i) raise funds to advance the necessary basic and operational research; and (ii) set policies, proposals, protocols, under the umbrella of the consortium.

If you are interested in participating, please submit a letter of interest to the Interim Steering Committee of the consortium briefly stating the extent of your interest in these areas, your experience and your association with leprosy field studies. A standard form and more information is available at: [http://www.kit.nl/biomedical_research/htm/leprosy_research_consortium.asp](http://www.kit.nl/biomedical_research/htm/leprosy_research_consortium.asp)

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Williams on her work in defining a partial \textit{M. leprae} transcriptome. Dr. Patrick J. Brennan, Colorado State University, Fort Collins, Colorado, U.S.A., presented his work on the genes encoding \textit{M. leprae} cell wall components. Last, Dr. Thomas P. Gillis, National Hansen’s Disease Programs Laboratory, discussed his research on the use of bioinformatics to search for potential vaccine and skin test antigen candidates.

This informative and timely symposium was attended by over 350 meeting participants and each presentation stimulated thoughtful discussion. In an effort to share the proceedings of this symposium with the readership of the \textit{International Journal of Leprosy} who were unable to attend the meeting, the four lecturers have here provided an overview of their presentations.

—Linda Adams

\textit{Chair, Division U (Mycobacteria)}
\textit{American Society for Microbiology}

\section*{ABSTRACTS}

\textbf{Britton, W. J.} On the vaccine trail: from Leprosy to Tuberculosis.

The control of leprosy has improved markedly since the introduction of multidrug therapy with dramatic falls in the prevalence of leprosy patients receiving antimicrobial therapy during the 1990’s. Despite this widespread implementation of MDT, the incidence of leprosy as measured by case detection rate has not yet fallen in major endemic countries. This justifies continuing research to understand the transmission, host response in protective immunity against \textit{Mycobacterium leprae}. Immunization to improve host response against \textit{M. leprae} infection will also be an important component in the long term control of leprosy.

\textbf{Current leprosy vaccines.} The antituberculosis vaccine, \textit{Mycobacterium bovis} bacille Calmette-guerin (BCG) has partial efficacy against clinical leprosy. In four randomized clinical trials, BCG stimulated a degree of vaccine efficacy (VE) ranging from 34\% in India to 80\% in Uganda. The prospective vaccine study in Malawi demonstrated a 52\% VE for BCG, with a further 50\% reduction in clinical leprosy following repeat BCG immunization \cite{1}. In addition, 9 case control studies have shown that past BCG immunization, as indicated by the presence of BCG a scar, was associated with approximately 50\% reduction in clinical leprosy (range 20 to 81\%). The impact of widespread BCG implementation on leprosy control is difficult to quantify, however, it is probable that BCG is one factor which has contributed to the decline in leprosy in some countries. The recent South India vaccine trial provided further evidence of the effectiveness of vaccines against clinical leprosy \cite{2}. In this study the addition of heat-killed \textit{M. leprae} to BCG resulted in improvement in the vaccine efficacy from 34 to 61\%. This is contrast to earlier studies in Malawi and Venezuela, which showed no benefit from the addition of heat-killed \textit{M. leprae} to BCG \cite{1}. An additional finding was that the cultivatable bacillus ICRC, probably a member of the \textit{M. avium-intracellulare} family, also conferred significant protection (VE, 65\%) when given as a dead mycobacterium. This provides further evidence that immunization with heterologous mycobacteria protects against \textit{M. leprae}.

\textbf{Subunit vaccines against leprosy.} The potential of subunit vaccines against leprosy was raised by the early studies of Gelber and Brennan, which showed that crude \textit{M. leprae} cell wall fractions and native cell wall-derived proteins protected against \textit{M. leprae} footpad infection in mice. More recently, Ngamying and colleagues showed that \textit{M. leprae} cytosol and membrane fractions protected against mouse footpad infection, but the cell wall skeleton of mycolyl-AG peptidoglycan from \textit{M. leprae} was not protective \cite{3}. Therefore, protein components are essential for effective subunit vaccines. The trail to determine which \textit{M. leprae} protein antigens induce effective immunity dates back to studies with monoclonal antibody-defined proteins in the 1980’s. These included the \textit{M. leprae} GroEs, GroEl and 70 kDa heat shock proteins, widely shared with other mycobacteria, and the \textit{M. leprae} 18 kDa protein, which contains \textit{M. leprae}-specific T cell epitopes but has a homologue in \textit{M. avium}. Subsequently, native proteins were purified from \textit{M. leprae}, including the cytoplasmic 10 kDa GroEs protein and the membrane-associated proteins MMPI and MMPII. Fractions of \textit{M. leprae} containing GroEs
and MMPI stimulated some protective effect in the mouse footpad model. More recently, a bioinformatics approach has been employed to define M. leprae homologues of M. tuberculosis antigens known to induce protective immunity against tuberculosis, such as the secreted proteins, Antigen 85B and ESAT-6.

Our own group has focused on the M. leprae 35 kDa (MMPI) as a subunit vaccine against leprosy. This protein was first recognized by M. leprae-specific monoclonal antibodies to conformational determinants on the protein, which are also the dominant epitopes for human leprosy sera. We cloned the gene for the M. leprae 35 kDa protein (10) and found it had no homologues in M. tuberculosis or BCG, but one was present in M. avium with 94% amino acid identity (5). Recombinant 35 kDa protein expressed in the rapidly growing M. smegmatis forms highly immunogenic multimers of >900 kDa. This protein is recognized across the leprosy spectrum, so that paucibacillary leprosy patients and contacts of leprosy patients develop a strong T cell response with low levels of antibody and multi-bacillary patients demonstrate a strong antibody response and weak T cell response (4). Further, the protein elicits delayed type hypersensitivity (DTH) in M. leprae sensitized guinea pigs.

DNA vaccines expressing the M. leprae or M. avium 35 kDa proteins induced protective immunity against M. leprae and M. avium infection in mice, which was equivalent to BCG in both cases (6, 7). This was accompanied by strong specific Interferon (IFN)-γ T cell responses, as well as high titre antibody responses to the conformational determinant on the protein. This establishes that immunization with a single antigen can be effective against experimental leprosy infection. Major antigens shared with M. tuberculosis and BCG may also induce protection when used as a subunit vaccines. For example, immunization with the M. tuberculosis antigen 85B as a DNA vaccine induced heterologous protection against M. leprae footpad infection in Swiss albino mice. (8).

Improving subunit vaccines against mycobacterial infections. Although subunit vaccines against leprosy infection show an equivalent protection to BCG in a mouse model, single protein or DNA vaccines against tuberculosis infection have generally been less effective than BCG in mice (9). Therefore, we and others have been examining ways of increasing the protective efficacy of subunit vaccines against mycobacterial infections. A number of cytokines were compared as adjuvants for DNA immunization and plasmid IL-12 was the most effective. Co-immunization with DNA-85B and plasmid IL-12 resulted in a rise in IFN-γ and T cell responses, a fall in specific antibody responses with increased protection against M. tuberculosis infection (9). Co-immunization with DNA-35 and plasmid IL-12 produced significantly greater protection against virulent M. avium infection than BCG (11). IL-12 was more effective than IL-18, another Th1-promoting cytokine, at improving DNA vaccine efficacy against mycobacterial infections (12).

We have also examined the interactions of subunit vaccines and BCG against M. tuberculosis infection. Priming with a DNA vaccine expressing the M. tuberculosis antigen 85B, followed by boosting with BCG, significantly improved the effective efficacy against M. tuberculosis to a level greater than that achieved with BCG alone (10). This may be due to focusing of the immune response against a dominant secreted antigen of M. tuberculosis. These findings demonstrate that protection against mycobacterial infections in experimental models is not limited to that achieved with BCG alone.

Challenges for new anti-leprosy vaccines. Although considerable progress has been made, there remain uncertainties in the understanding the complex host immunological response to mycobacteria and this influences the development of more effective anti-leprosy and anti-tuberculosis vaccines. First, the factors which determine the immunological dominance of antigens are still not resolved. Factors which may affect this include the quantity of mycobacterial protein and the timing of exposure. For example, the GroES protein is the major cytoplasmic protein in armadillo-derived M. leprae, and is a dominant antigen in host response to M. leprae (13). Secreted proteins, including antigen A85 complex, may be the first antigens encountered and appear to stimulate protective immunity against a number of mycobacteria (8, 10). There may
be intrinsic properties to certain proteins, such as the multimeric form of the 35 kDa \textit{M. leprae} protein, which contribute to their persistence in the phago-lysosome and so their antigenicity.

Second, the importance of species specificity in determining the protective efficacy of individual proteins is unclear. In fact, species-specific proteins may not be the optimal vaccine candidates, although they have obvious importance as a diagnostic reagents. Recent studies by Black and colleagues in Malawi (14) have demonstrated apparent cross-reactivity between the major antigens of \textit{M. leprae}, including the 35kDa and 18kDa proteins, and environmental mycobacterial species isolated in Malawi. These antigens may stimulate pre-existing T cell responses in infected subjects in endemic regions and this may blunt the apparent effectiveness of mycobacterial vaccines in that environment.

Third, the relative contribution of different T cell subsets to protective immunity may vary between different species of mycobacteria. Both CD4 and CD8 T cells appear to contribute to effective immunity against \textit{M. tuberculosis}, although the exact role of CD8 T cells inducing protection against \textit{M. leprae} infection has not been established.

Fourth, understanding the factors controlling of T cell memory, particularly in CD4 T cells is incomplete and this has major implications for subunit vaccines against mycobacterial infections. Although protein and DNA subunit vaccines can stimulate short term protective immunity against tuberculosis and leprosy in animal models, their ability to stimulate long term protection is yet to be determined. These subunit vaccines may prove most useful in boosting immunity established by BCG or other viable vaccines.

The second major challenge for new anti-leprosy vaccines are limitations of the models for testing protective efficacy. The dynamic range of the mouse footpad infection model is low, and it is difficult to measure increased effective efficacy above that achieved with BCG. The lack of immunological reagents and complexibility of the armadillo model mean that it is not currently applicable to testing vaccines. Further, the length of time for testing individual vaccines, currently 9 to 12 months, restricts the rate of progress. In addition, it is important to confirm that the vaccine antigens induce protective immunity in mice with differing genetic backgrounds to confirm their applicability in human populations.

The third and most important challenge for introduction of new anti-leprosy vaccines is the capacity to test these in endemic regions. Will it be possible to conduct another major human leprosy vaccine trial on the scale of the recent Indian vaccine study? This would require sufficient number of new cases and the extensive infrastructure required for such a study. Another approach would be to include leprosy in future tuberculosis vaccine trials. A number of candidate TB vaccines are moving into Phase I and Phase II clinical trials. If these are to be used in leprosy endemic countries, it is be important that they have an effective anti-leprosy component, either because the antigens are shared between \textit{M. tuberculosis} and \textit{M. leprae}, or \textit{M. leprae}-related components are added to the vaccine. This will require the design and conduct of the vaccine trials to measure the effects on leprosy as well as tuberculosis in leprosy-endemic regions.

Acknowledgement. Laboratory research has been funded by the National Health and Medical Research Council of Australia, the World Health Organization, the Co-operative Research Center for Vaccine Technology and the New South Wales Department of Health. I thank E. Martin, J. Triccas, U. Palendira and A. Kamath in Sydney and Dr. P. Roche, Anandaban Leprosy Hospital, Kathmandu, Nepal, for their collaboration and discussions. I am grateful to Dr. J. Velemner, Leprosy Mission International for his analysis of the effectiveness of BCG vaccines.

REFERENCES
The genome of *Mycobacterium leprae* has been completely sequenced and annotated. Approximately, 1604 open reading frames, encoding potentially functional proteins, and 1104 inactivated genes (pseudogenes) have been identified. However, the minimum gene set required for intracellular growth and survival (transcriptome) has not yet been defined. To address this, we have initiated studies to determine the potential transcriptome using RT-PCR and cross-species DNA microarray analysis using a comprehensive *M. tuberculosis* array using a commercially available oligonucleotide set (Operon Technologies, Alameda, CA) as a prelude to evaluating global gene expression using an *M. leprae* cDNA array, which is not currently available. For RT-PCR, RNA was obtained from two geographically distinct strains of *M. leprae* and cDNA was produced by reverse-transcription using random priming. Gene transcripts of interest were amplified from cDNA using PCR with primer sets flanking gene fragments of several potentially functional families of *M. leprae*. PCRs were initially characterized using DNA from *M. leprae* T-53 resultant PCR fragments were analyzed by gel electrophoresis. Cross-species DNA microarray analysis was accomplished using 5 μg total RNA from T-53 and 4089 and labeled with either Cy3 or Cy5 fluorochromes using RT. The labeled cDNAs will be hybridized to the slides, the slides will be washed and scanned using an Axon Scanner. The intensities of the two dyes at each spot will be quantified using the GenePix software package. Results of RT-PCR and cross-species microarray experiments demonstrated that genes encoding a variety of enzymes were transcribed in both strains. These include enzymes involved with folic acid synthesis, iron utilization, cofactor biosynthesis, gluconeogenesis, glycolysis, glyoxylate bypass, those associated with beta oxidation of fatty acids, degradation of phosphorous compounds, degradation of DNA, detoxification and virulence associated proteins, synthesis of mycolic acids, modification and maturation of ribosomes, synthesis of RNA, stress proteins, proteins of the SecA-dependent secretion pathway and 25 proteins containing secretion motifs or, and several proteins with unknown

functions. These data have provided us with the first insight into the transcriptome of *M. leprae* and further demonstrated the homogeneity of this species. It is anticipated that this analysis will help to identify a larger set of functional genes in *M. leprae* which will potentially help us to understand the minimal requirements for growth and replication of this pathogen. This information may lead to the identification of new drug targets, skin test antigens, and to identify factors that allow this pathogen to evade the immune system and destroy peripheral nerves.


Sequencing of the *Mycobacterium leprae* genome by S.T. Cole, *et al.* (http://www.nature.com/nature/v409/n6823/fig_tab/4091007a_F1.html) was a momentous event, comparable to the introduction of MDT (multiple drug therapy) in the 1980’s. Initial analysis indicated a genome size of about 3.3 megabases, a G/C content of 49.5% (these latter figures are to be compared to a size of about 4.4 megabases for the *Mycobacterium tuberculosis* H37Rv genome, a G/C content of 57.8%, only 1604 protein genes, 1116 pseudogenes, and hence a protein coding capacity of 49.5% (these latter figures are expected, since rhamnose is a component of the key diglycosyl-phosphoryl unit joining the mycolyl-arabinogalactan complex to peptidoglycan. Most of the known genes responsible for the synthesis of mannose, the PIMs (phosphatidylinositol mannosides), LM (lipomannan), and LAM (lipoarabinomannan), are present in *M. leprae*, such as *pmmA*, the Rv3256c homolog, *pmi*, *manB*, the Rv2609c homolog, *pimA*, Rv2611c homolog, and *pgsA*. However *pimB* is apparently missing, which requires new thinking on the mechanism of synthesis of PIMs/LM/LAM; we do know from chemical analysis that all three types of products are present in *M. leprae*. Likewise, most of the genes for mycolic acid synthesis, modification, and deposition are present in the *M. leprae* genome, such as *fasI*, *fabD*, *acpM*, *kasA*, *kasB*, *accD6*, *mabA*, *inhA*, *umaA2*, *mmaA4*, *mmaA1*, *fbpA*, *fbpB*, *fbpC*, and *fbpC2*. However, as noted initially by Cole, *et al.*, *umaA1*, *mmaA3*, and *mmaA2* are missing as whole genes, and this absence is exactly in accord with the absence of methoxymycolates in *M. leprae* as reported by several workers in the 1980’s.

The *pks* (polyketide synthase)-like genes of *Mycobacterium tuberculosis* responsible for the synthesis of the pthiocerol, phenolphthiocerol, and methyl branched fatty acids of DIM (dimycocerosyl pthiocerol) and the phenolic glycolipids (PGL) are receiving considerable current attention, since these products have been implicated in disease processes. In the case of *M. tuberculosis*, it has been demonstrated that disruption of the *pks10* and *pks7* genes which are clustered with *pks8*,
pks17, pks9 and pks11 resulted in mutants deficient in the synthesis of DIM. Similarly, the pks12 gene has also been implicated in synthesis of DIM. However, a careful analysis of the M. leprae genome indicates that pks10, pks7 and pks12 are pseudogenes; and pks 8, 17, 9, and 11 are absent.

An analysis of the intact pks genes of M. leprae from a different perspective, i.e., from the perspective of identification of the genes responsible for the synthesis of PGL-I, yields interesting information. The entire array of genes attributed to the synthesis of phthiocerol (ppsA–E) is located between ML2357 and ML2353. At a different location (ML0139) is the mas gene responsible for mycocerosic acid synthesis. The pks1 and pks15 of M. tuberculosis genes are fused as one gene in the M. bovis genome, which has been demonstrated to be involved in the synthesis of phenolphthiocerol, most likely a precursor of the M. bovis specific PGL. The M. leprae genome also contains the fused gene in concordance with the elaboration of its phenolic glycolipids. Elongation occurs with the ppsA–E cluster. Associated with ppsA–E are the genes fadD26 and drrA–C implicated in the attachment of mycocerosic acid and phthiocerol followed by transport through the membrane. Putative glycosyltransferases and methyltransferases possibly involved in the synthesis of the trisaccharide segment of PGL-I can be located in one cluster (ML0125-ML0130).

We have previously commented on the “cell wall gene cluster” of M. leprae characterized by the presence of embA–C implicated in arabinan synthesis, the fbp genes responsible for mycolic acid deposition, and glf and glfT involved in D-galactofuranose and D-galactan synthesis. A detailed comparison of gene arrangements in this cluster in the M. leprae and M. tuberculosis genomes demonstrates the absence of several genes (Rv13784-3788) from the M. leprae genome, one of which is a putative glycosyltransferase (Rv3786c). Additionally, a dedicated analysis of putative glycosyltransferases over the entire genome of M. leprae versus M. tuberculosis shows that several orthologs (e.g., Rv1312c, Rv0539, and Rv2957) representative of the glycosyltransferase families 1 and 2 are missing from M. leprae. Likewise, a cluster of at least 8 glycosyltransferases in the Rv1500 to Rv1526 region of the M. tuberculosis genome is missing from M. leprae. This evidence supports the chemical evidence for “stunted” or “truncated” versions of some polysaccharides, such as LAM, in M. leprae.

Thus, an analysis of the genome of M. leprae versus those of M. tuberculosis, M. bovis, and other Mycobacterium spp. supports the chemical analytical evidence of an intact but minimal cell wall in M. leprae.

Some relevant publications:


Gillis, T. The Use of Bioinformatics in Leprosy Research.

Bioinformatics encompasses all aspects of biological information acquisition and analysis, and combines the tools of computer science and biology with the aim of understanding biological significance. The combination of enhanced sequencing and computing power has allowed for unprecedented advances in assimilating huge amounts of raw data and the initiation of meaningful molecular modeling. Bioinformatic approaches are particularly attractive for aiding studies of M. leprae since many conventional biological tools are unavailable to investigators working with nonculturable agents.

Three major areas of bioinformatics (genomics, proteomics, and transcriptional profiling) have had and should continue to have an impact on our understanding of M. leprae and the disease it causes. Genomics has provided a working genetic blueprint for M. leprae allowing for comparisons with other mycobacterial genomes to assess M. leprae’s basic physiological capabilities, potential virulence factors and establish
molecular markers for drug resistance and strain variation. Newly developed strain identification markers using simple repeated DNA sequences should provide tools to investigate transmission patterns of leprosy and may help define risk factors involved in reinfection versus relapse of disease. Algorithms for predicting open reading frames and for categorizing location and function of *M. leprae* proteins have initiated basic studies on secreted proteins as well as proteins involved in nerve invasion and *M. leprae*-specific proteins potentially useful for detecting exposure to the leprosy bacillus through skin testing or serological testing.

Proteomic studies of the leprosy bacillus have been hampered by low protein yields from purified bacilli derived from infected animals, but have established a baseline profile of highly expressed proteins from *M. leprae*. Combining transcription profiling of the leprosy bacillus with proteomic studies may provide new insights into proteins previously lost during purification from infected host tissues and provide new antigens useful for studying immune responses during infection. In addition, newly discovered proteins can be tested for their ability to induce protective immunity and may constitute a new group of proteins with vaccine or diagnostic potential. Transcriptional profiling may allow investigators to study *M. leprae*’s gene expression at different stages of growth as well. For example, gene expression in growth-permissive cells, such as the macrophage and Schwann cell could differ and, therefore, may reveal aspects of *M. leprae*’s unique tissue tropism.

Functional genomics is the integration of predictive bioinformatics with validation through experimental biological analysis. This part of the equation remains a major challenge to workers in the leprosy field. Surrogate genetics to study *M. leprae* genes in cultivable mycobacteria as well as new approaches for “knocking in” genes to *M. leprae* are areas in need of research and development. Both approaches will benefit from bioinformatics and should continue to further our understanding of the leprosy bacillus in particular, and the host-parasite relationship in general.
THIRTY-EIGHTH U.S.-JAPAN TUBERCULOSIS-LEPROSY RESEARCH CONFERENCE

Public Health Research Institute
Newark, New Jersey

21–22 July 2003

Sponsored by the U.S.-Japan Cooperative Medical Sciences Program National Institute of Allergy and Infectious Diseases National Institutes of Health


With the recent completion and annotation of the genomes of *M. tuberculosis* and *M. leprae*, and additional sequence data from environmental mycobacteria (*M. avium*, *M. marinum*, and *M. smegmatis*), our ability to identify and test leprosy-specific antigens is much improved. Comparative genomic analysis of the *M. leprae* genome has identified 1604 open reading frames, as well as 1116 inactivated genes (pseudogenes), and up to 165 genes with no orthologue in *M. tuberculosis*. Through comparative genomic analysis of all mycobacterial databases, we have targeted 28 of these novel *M. leprae* genes for cloning and expression as recombinant proteins. This report characterizes our initial findings of five of these selected unique proteins, with a comparison of several other *M. leprae* proteins that have homologues in *M. tuberculosis*. Through cloning from *M. leprae* genomic DNA by PCR, all were produced as single recombinant proteins in *E. coli*. Our strategy was to produce polyclonal antisera against all of these single proteins, and then to use each antiserum to examine the relative amounts of the individual proteins in the native subcellular fractions of *M. leprae* (cytosol, membrane, and soluble cell wall antigens). In this way, we had previously shown the existence of native *M. leprae* ESAT-6 in the cell wall fraction in amounts significant enough to suggest that it could stimulate antibody or T cell responses in a natural infection. Indeed, several investigators have recently shown that leprosy patient PBMC respond well to both *M. leprae* ESAT-6 and CFP-10 proteins and peptides. Our initial analysis of the five unique *M. leprae* proteins by Western blot showed no detectable levels of any of these proteins in the native subcellular fractions. Nevertheless, by RT-PCR analysis of *M. leprae* cDNA isolated from two geographically different strains of *M. leprae* (Thai-53, originally isolated from a patient from Thailand, and strain 4089, originally isolated from a patient from Mexico), it appears that all of these genes have mRNA transcripts, and, thus, are potentially transcribed. However, further testing of these five recombinant proteins in *M. leprae*-sensitized guinea pigs did not reveal any detectable delayed type hypersensitivity (DTH) response. In addition, the proteins were examined by ELISA assay using leprosy patient sera (including sera from multibacillary lepromatous and paucibacillary tuberculoid patients). Responses to the unique antigens showed much lower responses overall, with the majority of the O.D. readings falling in the low to background level response range, consistent with the level of these antigens produced, while responses to well expressed antigens were much higher. It is possible that some of these novel proteins are just not that immunogenic. Alternatively, despite the lack of a significant antibody response, T cells from PBMC of leprosy patients may react better in IFN-γ assays to some of these unique proteins or their peptides, a possibility we are currently exploring. In light of our current findings, we should seriously question the strategy of choosing antigens for diagnostic purposes based on comparative genomics alone. It would appear from these results that some genes unique to *M. leprae*, although capable of generating a transcript, are not expressed in sufficient quantities in the bacillus itself to induce a meaningful immune response, and hence should not be pursued for diagnostic purposes. Alternative strategies for the selection of promising antigens will be discussed.

Up until now, the characterization of strain and lineage of *Mycobacterium leprae* isolates has been a great obstacle to studying the epidemiology of leprosy. In this post-genome era, the availability of a complete DNA sequence for *M. leprae* has provided an opportunity to target and analyze specific sites within the genome. Our approach for molecular typing of *M. leprae* includes the detection of variable number of short tandem repeat (STR) sequences, in different isolates. The specific goal of this research is to identify several polymorphic sites and combine them into a multiplex PCR-based molecular typing tool, which could be used on clinical biopsy samples collected in endemic parts of the world. We hope to develop a product (technique and reagents) that is portable, easy to apply and analyze, and inexpensive.

The first step, localization of regions of the genome within the sequenced Tamil Nadu (TN) strain containing a succession of single, di- and tri-nucleotide repeats, was accomplished by utilizing the search pattern program of the Leproma database (http://genolist.pasteur.fr/leproma/). A panel of 31 distinct sites in non-coding regions that contained 5 or more repeat units was identified. From these, several primary sites were selected. Primer sets were designed to flank the individual STR sites and elicit PCR products of incremental length to facilitate multiplexing in the future. A total of six *M. leprae* DNA sources (armadillo derived human clinical isolates) were used to search for polymorphisms by comparing the STR lengths. The PCR products were cloned to enable sequence confirmation of the entire amplified fragment, including the repeat region.

So far, we have confirmed repeat length polymorphisms in four of the DNA sources, and in all but one STR sequence. One of the AT repeat regions, showed as few as 25 bp and as many as 37 bp within the STR sequence of the various DNA sources when compared to the 37 bp sequence reported in the TN strain. Likewise, a different AT repeat sequence varied from 20 to 30 bp in the different DNA isolates while it is at 34 bp in the TN strain. A multi-C repeat sequence that is 20 bp in the TN strain ranged from 8 to 14 bp in the *M. leprae* isolates. Alternatively, a CG repeat sequence was found to have the same length as the TN strain, in all isolates tested.

Our findings clearly indicate that polymorphisms are present at various sites in the genomes of different strains of *M. leprae*, and consequently could be developed to track their lineage. Further emphasis will be placed on the use of fluorescent 5′ labeled primers for amplification, detection and fragment length analysis of the multiplexed products from the six DNA isolates on sequencing gels to improve throughput. The next phase will be expansion of the analysis to some of the remaining selected loci, followed by inclusion of a larger collection of DNA isolates. The final goal is to produce a fingerprinting system for *M. leprae* obtained from clinical isolates and to provide a tool for studying other aspects of leprosy such as transmission, virulence, drug resistance and relapse.

**Williams, D. L.** Gene Expression and Stability of mRNA in *Mycobacterium leprae*.

Evaluation of gene expression in *Mycobacterium leprae* under different experimental conditions should provide important insight into the physiology and therefore, the life-style of this noncultivable mycobacterium. Recently, we have identified mRNA transcripts for 194/200 genes analyzed by RT-PCR and the presence of polyadenylated mRNAs in 15/15 transcripts analyzed by oligodT priming of total RNA. Since polyadenylation in bacteria leads to mRNA stabilization under some conditions and degradation under other conditions, polyadenylation of mRNA in *M. leprae* may not be a useful indicator of mRNA stability. Nothing is known about the stability of mRNA in this slow growing pathogen. The purpose of the current study was to determine the relative stability of selected polyadenylated mRNAs in *M. leprae* after termination of transcription. To accomplish this, a suspension of mouse footpad-derived *M. leprae* in 7H12 medium was killed by subjecting bacteria to $5 \times 10^6$ rads of γ-radiation for 4 hr followed by rifampin (8 µg/ml final concentration) treatment to terminate mRNA transcription. RNA was purified from untreated bacteria (live) and the from dead bacteria stored at 33°C for 24, 48, 72 hr and...
1 week post treatment. The expression of five *M. leprae* genes from various gene families, previously found to be transcribed in the mouse foot pad (*rpoB*, *gyrA*, *folP1*, *hsp18*, and *sodA*), was determined using semiquantitative RT-PCR. Appropriate controls for DNA contamination were included for each template analyzed. The 16S rRNA PCR product from each template was used to normalize these data for template variations. Results demonstrated that transcripts for all genes studied were detected in the live bacteria for the entire experiment. In addition, transcripts for *rpoB*, *folP1*, and *hsp18* were observed up to one week post death, although transcript levels were lower than that of the untreated controls and progressively decreased with time. Transcripts for *gyrA* were observed up to 48 hr post treatment, although transcript levels were lower than those found in the live bacteria. Transcripts for *sodA* were not observed in the 24 hr treatment cDNA indicating that this mRNA is less stable than the rest of the mRNAs studied. These results demonstrated that the relative stability of the selected polyadenylated mRNAs varied suggesting that polyadenylation in *M. leprae* can not be used as an indicator of mRNA stability. Therefore, it is recommended that mRNA stability analysis be conducted on genes of interest prior to designing gene expression experiments for *M. leprae* because down regulation of the expression of a gene may not be detected in the background of a stable transcript encoded by that gene. In addition, the relatively short stability of the *sodA* transcripts suggests that further studies should be conducted to determine if mRNA is a suitable target for the development of rapid real time RT-PCR assay for the viability of *M. leprae*.

**Truman, R., Fontes, A., Suffys, P., Gillis, T.** Characterization of VNTR Polymorphisms in *Mycobacterium leprae*.

Differentiating mycobacteria at the subspecies (strain) level based on genomic diversity can be useful in understanding their evolution and for discriminating relationships between different clinical cases or laboratory strains. Early attempts to define strain variants of *M. leprae* have been of limited value because *M. leprae* appeared to show limited heterogeneity at the phenotypic and genomic levels. Insertion sequences are not abundant in *M. leprae* and, therefore, are not expected to provide sufficient intrusion into the genome to create variation useful for developing a strain typing system. Similarly, neither restriction fragment length polymorphism analysis (RFLP) nor 16S rDNA sequencing has identified diversity among *M. leprae* isolates from patients or natural animal hosts. More recently, various repeating elements have been shown to be effective in genotyping other highly conserved bacterial species. The completed sequence of the *M. leprae* genome reveals a number of such elements and some limited diversity already has been reported among a few clinical specimen within these repetitive sequences.

In Japan, limited variation in a 6 bp repeat in the *rpoT* gene was found among isolates from one region but it was absent among isolates in other locales. Somewhat greater diversity has been observed among *M. leprae* derived from a number of patient samples in the Philippines, where polymorphism was observed ranging from 10 to 37 copy numbers of a TTC (GAA) triplet repeat occurring in an intergenic segment downstream of pseudogene. Along with a few other repeating elements of unknown diversity, the significance of these variable number tandem repeat (VNTR) markers in *M. leprae* and their utility for epidemiological or clinical study has not yet been described.

We examined the diversity of GAA (same as TTC repeat described by Shin, et al.) repeats along with 3 other VNTR markers using a battery of clinical samples and *M. leprae* reference strains derived from patients and wild animals in different geographic regions. To help assess the suitability of these VNTR markers for laboratory and community based studies, we also examined the stability of the reference strain VNTR genotype with passage in different animals under varied conditions and in different tissues.

**Lahiri, R., Randhawa, B., and Krahenbuhl, J.** Further definition of viability of *Mycobacterium leprae* as a research resource.
A rapid and reliable method to compare viability between two suspensions of *Mycobacterium leprae* is needed. We have shown previously that a radiorespirometry method, which assesses the metabolic activity of *M. leprae*, compares well with the mouse footpad assay. In this study, we tested a two-color (Syto9 and Propidium iodide) fluorescence assay to determine if a rapid direct count viability staining can be applied to *M. leprae*. A variety of experimental conditions were employed to assess this “viability stain.” We also applied both radiorespirometry and viability staining on extracellular and intracellular *M. leprae* treated with different known antimycobacterial drugs to assess the reliability of these two methods taken together, in screening anti-leprosy drugs. We used Rifampin, Clofazamine, Dapsone, Ofloxacin, and Minocycline, on axenic and intracellular cultures of *M. leprae*. We found that the intracellular model of drug testing was more efficient in detecting anti bacterial effect of drugs in *M. leprae* than axenic cultures.

Matsuoka, M., Liangfen, Z., and Budiawan, T. Analysis of leprosy transmission based on genotyping.

*Mycobacterium leprae* isolates were divided into two groups by the polymorphism in the rpoT gene. Geographic distribution of the rpoT genotype of *Mycobacterium leprae* in Latin American countries was investigated in connection with human prehistoric migration. All *M. leprae* isolates from Peru and Paraguay showed three tandem repeats of 6 bp. On the contrary, 25 out of 27 isolates from Mexico revealed four tandem repeats. It was assumed that the leprosy was carried into these countries by different groups of ancient Mongoloids migrated to Latin America at different periods.

Genotyping by TTC repeat polymorphism was applied for epidemiological analysis of leprosy transmission. *M. leprae* on the nasal mucous membrane of villagers showed variety of TTC repeat numbers. *M. leprae* genotype of four leprosy cases in two houses was examined. *M. leprae* isolates from a couple of the father and a son showed the same TTC genotype but the father and the son in another house were infected by different *M. leprae* distinguished from TTC repeat. The findings suggested the transmission of the bacilli might be from various infectious sources, though it is generally believed the main infectious source is multi-bacillary patients and household contact with such patient has the highest risk of being infected. Infectious sources other than multi-bacillary patients in the household would be important for leprosy control especially in endemic countries. The results indicated the existence of the infectious source other than patients in the household.

Ohyama, H., Takeuchi, K., Yamada, H., Uemura, Y., and Matsushita, S. SNPs on IL-12 receptor gene associated with the susceptibility to leprosy.

Activated T cells from lepromatous leprosy patients are likely to produce low amounts of IFN-gamma even in the presence of IL-12. The objective of this study is to reveal the low productivity of IFN-gamma in leprosy patients from an immunogenetical viewpoint. The polymorphism of 5′ flanking regions of both *IFNG* and *IL-12RB2* were determined to compare the allele frequencies between patients and healthy donors, using direct sequencing technique. The results of the study are as follows. i) No polymorphism was detected in the promoter region of *IFNG*. ii) Several SNPs were detected in the 5′ flanking region of *IL-12RB2*, and SNPs located at the positions, −1035, −1023, −650 and −464 were more frequently detected in LL patients than in TT patients.

These results suggest that the polymorphism in the 5′ flanking region of *IL12RB2* may be implicated to determine disease form of leprosy.
Leprosy or Hansen’s disease is a chronic infectious disease caused by the Mycobacterium leprae. The skin and nervous manifestations of the disease present a singular clinical picture that is easily recognized. After India, Brazil still is the second country with the greatest number of cases in the world. Around 94% of the known cases and 94% of the new cases reported in America, come from Brazil. The disease presents itself in two well-defined stable and opposite poles (lepromatous and tuberculoid) and two unstable groups (indeterminate and dimorphic). The spectrum of presentation of the disease may also be classified as: tuberculoid tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL) and lepromatous lepromatous (LL). The finding of acid fast bacillus in tissue is the most useful method of diagnosis. The effective treatment of leprosy includes the use of specific therapy, suppression of lepra reactions, prevention of physical incapacity, and physical and psychosocial rehabilitation. Chemotherapy with rifampin, dapsone, and clofazimine have produced very good results and the control of the disease in Brazil in the foreseeable future is likely.—Author’s Abstract


Mycobacterium marinum is a pathogenic mycobacterial species that is closely related to Mycobacterium tuberculosis and causes tuberculosis-like disease in fish and frogs. We infected the fruit fly Drosophila melanogaster with M. marinum. This bacterium caused a lethal infection in the fly, with a 50% lethal dose (LD(50)) of 5 CFU. Death was accompanied by widespread tissue damage. M. marinum initially prolifer-
ated inside the phagocytes of the fly; later in infection, bacteria were found both inside and outside host cells. Intracellular M. marinum blocked vacuolar acidification and failed to colocalize with dead Escherichia coli, similar to infections of mouse macrophages. M. marinum lacking the mag24 gene were less virulent, as determined both by LD(50) and by death kinetics. Finally, in contrast to all other bacteria examined, mycobacteria failed to elicit the production of antimicrobial peptides in DROSOPHILA: We believe that this system should be a useful genetically tractable model for mycobacterial infection.—Authors’ Abstract


Although Crohn’s disease is considered to be autoimmune in origin, there is increasing evidence that it may have an infectious cause. The most plausible candidate is Mycobacterium avium subspecies paratuberculosis (MAP). Intriguingly, Koch’s postulates may have been fulfilled for MAP and Crohn’s disease, even though they still have not been met for Mycobacterium leprae and leprosy. In animals MAP causes Johne’s disease, a chronic wasting intestinal diarrhoeal disease evocative of Crohn’s disease. Johne’s disease occurs in wild and domesticated animals, including dairy herds. Viable MAP is found in human and cow milk, and is not reliably killed by standard pasteurisation. MAP is ubiquitous in the environment including in potable water. Since cell-wall-deficient MAP usually cannot be identified by Ziehl-Neelsen staining, identification of MAP in human beings requires culture or detection of MAP DNA or RNA. If infectious in origin, Crohn’s disease should be curable with appropriate antibiotics. Many studies that argue against a causative role for MAP in Crohn’s disease have used antibiotics that are inactive against MAP. However, trials that include macrolide antibiotics indicate that a cure for Crohn’s disease is possible. The necessary length of therapy remains to be determined. Mycobacterial diseases have protean clinical manifestations, as does Crohn’s disease. The necessity of stratifying Crohn’s disease into two clinical manifestations (perforating and non-perforating) when interpreting the results of antibiotic therapy is discussed. Rational studies to evaluate appropriate therapies to cure Crohn’s disease are proposed.—Author’s Abstract


Quantitative suspension and carrier tests were used to compare the activity of Perasafe and Cidex against Mycobacterium tuberculosis, Mycobacterium avium-intracellulare, Mycobacterium fortuitum, and Mycobacterium chelonae. The interference of an organic load, and of hard water was also considered. Both agents achieved reductions exceeding 10(5)-fold within 20 and 30 min for all the strains tested. Perasafe is thus mycobactericidal and a viable alternative to Cidex for intermediate or high-level disinfection.—Authors’ Abstract

Chemotherapy


The emergence of multi-drug resistant tuberculosis, coupled with the increasing overlap of the AIDS and tuberculosis pandemics has brought tuberculosis to the forefront as a major worldwide health concern. In an attempt to find new inhibitors of the enzymes in the essential rhamnose biosynthetic pathway, a virtual library of 2,3,5 trisubstituted-
4-thiazolidinones was created. These compounds were then docked into the active site cavity of 6′hydroxyl; dTDP-6-deoxy-D-xylo-4-hexulose 3,5-epimerase (RmlC) from *Mycobacterium tuberculosis*. The resulting docked conformations were consensus scored and the top 5% were slated for synthesis. Thus far, 94 compounds have been successfully synthesized and initially tested. Of those, 30 (32%) have ≥50% inhibitory activity (at 20 microM) in the coupled rhamnose synthetic assay with seven of the 30 also having modest activity against whole-cell *M. tuberculosis*. —Authors’ Abstract


In *vitro* screening of thiacetazone derivatives indicated that two derivatives, SRI-286 and SRI-224, inhibited a panel of 25 *Mycobacterium avium* complex (MAC) isolates at concentrations of 2 micro g/ml or lower. In mice, SRI-224 and thiacetazone had no significant activity against the MAC in livers and spleens, but treatment with SRI-286 resulted in significant reduction of bacterial loads in livers and spleens. A combination of SRI-286 and moxifloxacin was significantly more active than single drug regimens in liver and spleen. —Authors’ Abstract


Genomic technologies have the potential to greatly increase the efficiency of the drug development process. As part of our tuberculosis drug discovery program, we used DNA microarray technology to profile drug-induced effects in *Mycobacterium tuberculosis*. Expression profiles of *M. tuberculosis* treated with compounds that inhibit key metabolic pathways are required as references for the assessment of novel antimycobacterial agents. We have studied the response of *M. tuberculosis* to treatment with the mycolic acid biosynthesis inhibitors isoniazid, thiolactomycin, and triclosan. Thiolactomycin targets the beta-ketoacyl-acyl carrier protein (ACP) synthases KasA and KasB, while triclosan inhibits the enoyl-ACP reductase InhA. However, controversy surrounds the precise mode of action of isoniazid, with both InhA and KasA having been proposed as the primary target. We have shown that although the global response profiles of isoniazid and thiolactomycin are more closely related to each other than to that of triclosan, there are differences that distinguish the mode of action of these two drugs. In addition, we have identified two groups of genes, possibly forming efflux and detoxification systems, through which *M. tuberculosis* may limit the effects of triclosan. We have developed a mathematical model, based on the expression of 21 genes, which is able to perfectly discriminate between isoniazid-, thiolactomycin-, or triclosan-treated *M. tuberculosis*. This model is likely to prove invaluable as a tool to improve the efficiency of our drug development programs by providing a means to rapidly confirm the mode of action of thiolactomycin analogues or novel InhA inhibitors as well as helping to translate enzyme activity into whole-cell activity. —Authors’ Abstract


Here, we report the antimycobacterial activity of NCX 976, a new molecule obtained adding a NO moiety to the fluoroquinolone ciprofloxacin, on *Mycobacterium tuberculosis* H37Rv strain, both in a cell-free model and in infected human macrophages. Unlike unaltered ciprofloxacin, NCX976 displayed a marked activity also

The use of gatifloxacin (GAT) in combination with ethionamide (ETA) with or without pyrazinamide (PZA) for a 12-week treatment period followed by an 8-week observation period was evaluated in a model of tuberculosis in mice. Mice treated with GAT at 300 mg/kg of body weight in combination with ETA (25 mg/kg) for 5 days per week had sterile lungs, whereas mice treated with GAT (100 mg/kg) and ETA (25 mg/kg) had about 10 CFU/lung; however, there was regrowth of the organisms in both groups at the end of the observation period. When PZA (450 mg/kg 5 days per week) was added to the high-dose GAT-ETA regimen, no viable mycobacteria were present after the 8-week observation period. GAT in combination with ETA and PZA has great promise for the treatment of tuberculosis.—Authors’ Abstract


Thalidomide has recently shown considerable promise in the treatment of a number of conditions, such as leprosy and cancer. Its effectiveness in the clinic has been ascribed to wide-ranging properties, including anti-TNF-alpha, T-cell costimulatory and antiangiogenic activity. Novel compounds with improved immunomodulatory activity and side effect profiles are also being evaluated. These include selective cytokine inhibitory drugs (SelCIDs), with greatly improved TNF-alpha inhibitory activity, and immunomodulatory drugs (IMiDs) that are structural analogs of thalidomide, with improved properties. A third group recently identified within the SelCID group, with phosphodiesterase type 4-independent activity, is in the process of being characterized in laboratory studies. This review describes the emerging immunological properties of thalidomide, from a historical context to present-day clinical applications, most notably in multiple myeloma but also in other cancers, inflammatory disease, and HIV. We also describe the laboratory studies that have led to the characterization and development of SelCIDs and IMiDs into potentially clinically relevant drugs. Early trial data suggest that these novel immunomodulatory compounds may supercede thalidomide to become established therapies, particularly in certain cancers. Further evidence is required, however, to correlate the clinical efficacy of these compounds with their known immunomodulatory, antiangiogenic, and antitumor properties.—Authors’ Abstract


AIM: To evaluate the response rate to antimycobacterial drug therapy in patients with cystic fibrosis (CF) suffering from infection by non-tuberculous mycobacteria (NTM). METHODS: Ten patients, aged 10–34 yrs, out of 180 CF patients, were diagnosed with NTM disease. They had been regularly checked and examined for pulmonary symptoms, and had had chest X-rays and sputum cultures (including for mycobacteria) performed. One additional 36-yr-old female received her CF diagnosis soon after the NTM diagnosis. RESULTS: Mycobacterium avium-intracellulare complex (MAC) was found in 10 out of 11 patients and M. kansasii in 1 patient. Treatment with antimycobacterial drugs resulted in clinical improvement (weight gain or stabilization of weight and/or improved or stabilized lung function in 8 out of 11 patients) and mycobacterial culture turned negative in 10 out of 1. CONCLUSION: Promising results may be associated with early intervention with antimycobacterial therapy in CF patients.—Authors’ Abstract

OBJECTIVE: The long-term safety of therapeutic agents that neutralize tumor necrosis factor (TNF) is uncertain. Recent evidence based on spontaneous reporting shows an association with active tuberculosis (TB). We undertook this study to determine and describe the long-term safety of 2 of these agents, infliximab and etanercept, in rheumatic diseases based on a national active-surveillance system following the commercialization of the drugs. METHODS: We analyzed the safety data actively collected in the BIOBADASER (Base de Datos de Productos Biologicos de la Sociedad Espanola de Reumatologia) database, which was launched in February 2000 by the Spanish Society of Rheumatology. For the estimation of TB risk, the annual incidence rate in patients treated with these agents was compared with the background rate and with the rate in a cohort of patients with rheumatoid arthritis (RA) assembled before the era of anti-TNF treatment. RESULTS: Seventy-one participating centers sent data on 1578 treatments with infliximab (86%) or etanercept (14%) in 1540 patients. Drug survival rates (reported as the cumulative percentage of patients still receiving medication) for infliximab and etanercept pooled together were 85% and 81% at 1 year and 2 years, respectively. Instances of discontinuation were essentially due to adverse events. Seventeen cases of TB were found in patients treated with infliximab. The estimated incidence of TB associated with infliximab in RA patients was 1893 per 100,000 in the year 2000 and 1113 per 100,000 in the year 2001. These findings represent a significant increased risk compared with background rates. In the first 5 months of 2002, after official guidelines were established for TB prevention in patients treated with biologics, only 1 new TB case was registered (in January). CONCLUSION: Therapy with infliximab is associated with an increased risk of active TB. Proper measures are needed to prevent and manage this adverse event.—Authors’ Abstract


Versatile synthesis of the teratogenic, TNFalpha-modulatory, and antiangiogenic thalidomide analogue 2-(2,6-dioxopiperidine-3-yl)phthalimidine (1) and its direct antiangiogenic properties are described. With thalidomide or thalidomide derivatives as precursors, the synthesis involved either carbonyl reduction/thiation-desulfurization or carbonyl reduction/acyliminium ion reduction protocols. Compared to earlier studies with thalidomide, which was only active with microsomal treatment, 1 exhibited marginal inhibitory activity in the rat aortic ring assay, thereby demonstrating the requirement for metabolic activation.—Authors’ Abstract


Thalidomide is known to be effective in the treatment of a number of conditions, including leprosy and various cancers. The exact mechanisms of action remain unclear although these are known to include anti-tumour necrosis factor (TNF)-alpha, T cell costimulatory, anti-angiogenic and anti-tumour activities. However, thalidomide is being superceded by novel structural derivatives which have been designed to have improved immunomodulatory activity and side effect profiles. These are currently being characterised and some are entering the clinic in phase I/II studies. One novel group of structural analogues are classified as the
Immunomodulatory Drugs (IMiDs). This review describes the emerging immunological, anti-angiogenic and direct anti-tumour properties of thalidomide and the characterisation and clinical application of its IMiD analogues. We describe the laboratory studies which have led to the characterisation and development of IMiDs into potentially clinically relevant drugs. Early trial data suggests that these compounds may themselves become established therapies, particularly in certain cancers. Furthermore, ongoing studies will determine how best to apply these compounds to the appropriate clinical settings. We will describe the various clinical studies of lead compounds that are in progress and speculate as to the potential and future development of these exciting compounds.—Authors’ Abstract


OBJECTIVE: To review published data on thalidomide, with emphasis on current knowledge about mechanism of action, new and/or potential dermatologic and non-dermatologic therapeutic applications, well-known and emerging adverse effects, and current indications for its safe use. DATA SOURCES: Review articles, in vitro research studies, references from retrieved articles, case reports, and clinical trials were identified from a computerized literature search using MEDLINE and OVID (1966–January 2003) and on the Cochrane Clinical Trials Register (January 2003). Information available from meetings’ abstract books, Internet, or pharmaceutical companies was also considered. STUDY SELECTION AND DATA EXTRACTION: All articles identified as relevant, including those from non-English literature, were considered in an attempt to provide to the reader both the theoretical basis and practical guidelines for thalidomide pharmacotherapy. DATA SYNTHESIS: Thalidomide has hypnosedative, antiangiogenic, anti-inflammatory, and immunomodulatory properties. Moreover, it has been shown to selectively inhibit the production of tumor necrosis factor-alpha and reduce the expression of various integrin receptors on the membrane of leukocytes and other cell types in a dose-dependent fashion. Controlled trials demonstrated the efficacy of thalidomide in a number of diseases, including erythema nodosum leprosum, lupus erythematosus, aphthosis, graft-versus-host disease, prurigo nodularis, and actinic prurigo. Single case reports or studies in small series have also suggested a possible role for thalidomide in numerous other dermatologic and nondermatologic disorders. Possibly severe and sometimes irreversible risks related to the clinical use of thalidomide include teratogenicity and neurotoxicity. CONCLUSIONS: Although teratogenicity and neurotoxicity are significant adverse effects requiring cautious use, thalidomide is an effective therapeutic modality in a variety of difficult-to-treat disorders and, providing careful selection of patients, should offer an acceptable risk-to-benefit ratio.—Authors’ Abstract

Clinical Sciences


We describe a patient with relapsing B chronic lymphocytic leukemia who developed systemic bacille Calmette-Guerin infection (BCGitis) after administration of alemtuzumab (Campath-1H).—Authors’ Abstract

We report 2 cases of Lucio’s phenomenon, a rare, aggressive, occasionally fatal type 2 reaction occurring in the diffuse nonnodular type of lepromatous leprosy. The clinical diagnosis of Lucio’s phenomenon is difficult, and there are no known predictive or prognostic factors. Despite institution of aggressive treatment after diagnosis, our 2 cases had fatal outcomes.—Authors’ Abstract


A new diagnosis of borderline lepromatous leprosy was established in a man who had immigrated to Kentucky from Mexico. He was placed on a World Health Organization treatment regimen consisting of dapsone, clofazimine, and rifampin. The biology of leprosy, its diagnosis, treatment, and worldwide impact are reviewed. Because of the potential for highly mobile populations to export endemic diseases, Kentucky physicians must expand their lists of differential diagnoses.—Authors’ Abstract


Two patients showing abnormal fluorine-18-fluorodeoxyglucose (FDG) uptake due to Mycobacterium avium complex (MAC) infection are presented. Intense focal FDG uptake in the lung field could have been caused by an infectious disease such as MAC. This should be considered as a possibility when FDG whole-body scans of patients with pulmonary nodules are interpreted. To our knowledge, this is the first description of an FDG-positron emission tomography (FDG-PET) image of MAC infection of the lung.—Authors’ Abstract


Fatal agranulocytosis in an Indian male receiving 100 mg of dapsone daily, hospitalized for mid-borderline leprosy in type I reaction with triple nerve paralysis is reported. Various case reports concerning dapsone-induced agranulocytosis are reviewed.—Authors’ Abstract


We report the results of laser in situ keratomileusis (LASIK) in a 51-year-old woman with subsequent mycobacterial keratitis diagnosed by staining with acid-fast and fluorochrome methods, a technique known to have good sensitivity and specificity for mycobacteria. A rapid diagnosis was made without waiting for cultures, and treatment was instituted, including tapering of topical steroids and appropriate antibiotic therapy. The result was preservation of the LASIK flap and a favorable visual outcome at 6 months.—Authors’ Abstract


PURPOSE: To determine the magnitude of ocular complications that present in incident cases of relapsed borderline lepromatous (BL) and lepromatous leprosy (LL) patients. METHOD: From 1991 to 1997, all new BL and LL patients who had relapsed from an earlier disease, detected by active case finding in the geographically defined area of Gudiyattam taluk, were invited for ocular examination after their leprosy status was confirmed clinically and histopathologically. RESULTS: Sixty relapsed lepromatous patients, 45 male and
15 females, were examined. Fifty-two patients had relapsed after receiving only dapsone mono-therapy, 4 after receiving paucibacillary multi-drug therapy (PB-MDT) preceded by dapsone mono-therapy and 4 after only PB-MDT. Three (5%) patients had lagophthalmos, 1 (1.6%) patients each had ectropion and trichiasis, 32 (53%) patients had impaired corneal sensation in both eyes, 2 (3.3%) patients each had corneal opacity (associated with reduced vision), corneal nerve beading, punctate keratitis, keratic precipitates, and irisatrophy, 4 (6.6%) patients had cataract associated with reduced vision. Lagophthalmos was associated with increased duration of the disease \( (p = 0.009) \), Grade II deformity \( (p = 0.001) \), punctate keratitis \( (p <0.001) \) and cataract \( (p <0.001) \). Beaded corneal nerves were associated with lepromatous leprosy \( (p <0.001) \) and high mycobacterial infection \( (p = 0.05) \). Patients whose initial disease was categorised as BL and LL had greater impairment of vision \( (p = 0.037) \), more iris atrophy \( (p = 0.013) \), increased keratic precipitates \( (p = 0.013) \) and more corneal nerve beading \( (p = 0.013) \), when compared with the group comprising Tuberculoid-tuberculoid (TT), Borderline-tuberculoid (BT) and Intermediate (IND).

CONCLUSION: This first report on ocular complications in relapsed lepromatous patients demonstrates that general and leprosy-related ocular complications were responsible for reduced vision. Lagophthalmos was associated with increased duration of the disease \( (p = 0.009) \), Grade II deformity \( (p = 0.001) \), punctate keratitis \( (p <0.001) \) and cataract \( (p <0.001) \). Beaded corneal nerves were associated with lepromatous leprosy \( (p <0.001) \) and high mycobacterial infection \( (p = 0.05) \). Patients whose initial disease was categorised as BL and LL had greater impairment of vision \( (p = 0.037) \), more iris atrophy \( (p = 0.013) \), increased keratic precipitates \( (p = 0.013) \) and more corneal nerve beading \( (p = 0.013) \), when compared with the group comprising Tuberculoid-tuberculoid (TT), Borderline-tuberculoid (BT) and Intermediate (IND).

CONCLUSION: This first report on ocular complications in relapsed lepromatous patients demonstrates that general and leprosy-related ocular complications occur in these patients. However, they are not in excess of those reported in other leprosy groups. Borderline and lepromatous leprosy patients tend to have had more ocular complications than patients with tuberculoid leprosy.—Authors’ Abstract


Cutaneous leishmaniasis, leprosy, and tuberculosis are caused by intracellular pathogens whose development depends on impaired cell-mediated immunity. We report an exceptional triple association of American cutaneous leishmaniasis, lepromatous leprosy, and pulmonary tuberculosis in a man with no recognized immunodeficiency. Normal immunological assessment of the interferon-gamma pathway does not support the hypothesis of a genetic defect in any of the genes involved in the T helper (Th)-1 cytokine cascade in this patient. Unresponsiveness to interleukin (IL)-12 of his T cells after stimulation with \textit{Leishmania guyanensis}, \textit{Mycobacterium bovis} bacille Calmette-Guerin, and \textit{Mycobacterium leprae} antigens suggested the inability to mount an appropriate Th cell response to upregulate the IL-12 receptor expression.—Authors’ Abstract


BACKGROUND: Mycobacterial keratitis is a rare complication following LASIK but can lead to an extremely unfavourable outcome. The diagnosis and treatment is often delayed due to confusion with other entities including diffuse lamellar keratitis and poor clinical outcomes with flap amputation and/or keratoplasty are often the case. PATIENT AND METHODS: We report the results of LASIK in a 51-year-old woman with subsequent early-diagnosed mycobacterial keratitis and compared this case to treatments and outcomes reported in the literature. RESULTS: The patient presented 10 days following LASIK with a white focal infiltrate in the stromal interface. The flap was lifted and cultures from the stromal bed and the reverse of the flap were obtained and the interface irrigated. The patient was treated with topical antibiotics (ciprofloxacin 0.3%, amikacin 2.5%, clarithromycin 40 mg/ml and tobramycin 15
mg/ml) for 8 weeks and at the most recent follow-up she had a visual acuity of 1.25.

**CONCLUSION:** In a large number of published cases in the literature the flap had to be amputated and/or corneal transplants were necessary. Early diagnosis and treatment however, are essential to successfully treat post-LASIK keratitis. Therefore the patients should be followed up carefully in the early postoperative period.—Authors’ Abstract

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A total of 2026 leprosy patients of the National Sorokdo Hospital was examined their intestinal parasites by cellophane thick smear method in January 1983. The egg positive cases of *Taenia* spp. were treated with bithionol and the segments of Taenia were collected for species identification. The results were as follows: 1. Total egg positive rate of any kind helminth was 78.2% and cumulative total was 85.2%. The egg positive rate for each helminth was as follow; *Taenia* spp. 3.4%, *Ascaris lumbricoides* 4.5%, *Trichuris trichiura* 72.1%, *Clonorchis sinensis* 2.8% and other 0.05%. 2. A total of 66 Taenia egg positive cases was treated; out of them proglottids of Taenia were collected from 26 cases. All of the collected worms were identified as *T. saginata*. The results revealed significantly high egg positive rate of *T. trichiura*. However, *A. lumbricoides* was found to be controlled considerably by repeated chemotherapy during past 3 years. If chemotherapeutic agent is replaced with oxantel-pyrantel tablet, better result is expected. No clue was found for prevalence of *T. solium* from both human and the pig in the island.—Authors’ Abstract

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**Nakayama, S., Uesaka, Y., Kunimoto, M., Mikata, T., Shimizu, J., and Ishii, N.** [The painful multiple mononeuropathy of acute onset in the left arm which was diagnosed as leprous neuropathy]. Rinsho Shinkeigaku. 43(5) (2003) 265–269. [Article in Japanese]

A 31-year-old man from Myanmar with leprous neuropathy was reported. The progress of the disease was subacute but the painful symptom at the time of the onset was acute. Multiple mononeuropathy was diagnosed by the biopsy findings of the left superficial radial nerve. He was admitted to our hospital with the complaint of the weakness of his left hand and fingers which were very painful and got worse in several weeks. Motor palsy was observed in his left ulnar, median, and radial nerves, and there was the hypesthesia or anesthesia in his left hand, forearm and the medial side of his left upper arm. On nerve conduction studies, the amplitudes of CMAP and SNAP severely diminished or not detected. The pattern was compatible with multiple mononeuropathy. The biopsy of the left superficial radial nerve was performed. The pathological findings were the destruction of nerve fascicles, replacement of nerve fibers with inflammatory cells, and *Mycobacterium leprae* was found with the specific stain. These findings confirmed the diagnosis of the leprous neuropathy. Leprous neuropathy is one of the commonest causes of infectious neuropathy in the world, especially in Southeast Asia. These days many foreign workers from that area are staying in Japan, and the chances to see the disease are increasing. We have to recognize leprous neuropathy as a candidate for the multiple mononeuropathy of acute onset with painful dysesthesia similar to vascular neuropathy.—Authors’ Abstract

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**Anes, E., Kuhnel, M. P., Bos, E., Moniz-Pereira, J., Habermann, A., and Griffiths, G.** Selected lipids activate phago-

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Pathogenic mycobacteria such as Mycobacterium tuberculosis and Mycobacterium avium facilitate disease by surviving intracellularly within a potentially hostile environment: the macrophage phagosome. They inhibit phagosome maturation processes, including fusion with lysosomes, acidification and, as shown here, membrane actin assembly. An in vitro assay developed for latex bead phagosomes (LBPs) provided insights into membrane signalling events that regulate phagosome actin assembly, a process linked to membrane fusion. Different lipids were found to stimulate or inhibit actin assembly by LBPs and mycobacterial phagosomes in vitro. In addition, selected lipids activated actin assembly and phagosome maturation in infected macrophages, resulting in a significant killing of M. tuberculosis and M. avium. In contrast, the polyunsaturated sigma-3 lipids behaved differently and stimulated pathogen growth. Thus, lipids can be involved in both stimulatory and inhibitory signalling networks in the phagosomal membrane.—Authors’ Abstract


We have previously shown that young adults living in a rural area of northern Malawi showed greater gamma interferon (IFN-gamma) responses to purified protein derivatives (PPD) prepared from environmental mycobacteria than to PPD from Mycobacterium tuberculosis. In order to define the mycobacterial species to which individuals living in a rural African population have been exposed and sensitized, we tested T-cell recognition of recombinant and purified antigens from M. tuberculosis (38 kDa, MPT64, and ESAT-6), M. bovis (MPB70), M. bovis BCG (Ag85), and M. leprae (65 kDa, 35 kDa, and 18 kDa) in >600 non-M. bovis BCG-vaccinated young adults in the Karonga District of northern Malawi. IFN-gamma was measured by enzyme-linked immunosorbent assay (ELISA) in day 6 supernatants of diluted whole-blood cultures. The recombinant M. leprae 35-kDa and 18-kDa and purified native M. bovis BCG Ag85 antigens induced the highest percentages of responders, though both leprosy and bovine tuberculosis are now rare in this population. The M. tuberculosis antigens ESAT-6 and MPT64 and the M. bovis antigen MPB70 induced the lowest percentages of responders. One of the subjects subsequently developed extrapulmonary tuberculosis; this individual had a 15-mm-diameter reaction to the Mantoux test and responded to M. tuberculosis
PPD, Ag85, MPT64, and ESAT-6 but not to any of the leprosy antigens. We conclude that in this rural African population, exposure to *M. tuberculosis* or *M. bovis* is much less frequent than exposure to environmental mycobacteria such as *M. avium*, which have antigens homologous to the *M. leprae* 35-kDa and 18-kDa antigens. *M. tuberculosis* ESAT-6 showed the strongest association with the size of the Mantoux skin test induration, suggesting that among the three *M. tuberculosis* antigens tested it provided the best indication of exposure to, or infection with, *M. tuberculosis*—Authors’ Abstract


The objective of the study was to identify *Mycobacterium leprae*-specific immunogenic peptides for the development of a skin test reagent. Such a reagent is required for the detection of *M. leprae* infection and possibly for the diagnosis of patients with active leprosy. For this purpose, we analyzed the *in vitro* responses of human peripheral blood mononuclear cell (PBMCs) to peptides from the 35 kDa protein of *M. leprae*. This protein is of interest since it has no homologue within the *Mycobacterium tuberculosis* complex, although it has a homologue in *Mycobacterium avium*. The subjects enrolled in the study were paucibacillary (PB) and multibacillary (MB) leprosy patients, healthy contacts, and non-contacts. Seventy-three PB and 124 MB leprosy patients were recruited from four leprosy clinics in Thailand. Fifty-seven healthy contacts were household contacts. Twenty non-leprosy contacts had no family history of or exposure to leprosy. PBMCs from individuals were tested for stimulation with 12 overlapping peptides from the *M. leprae* 35 kDa protein using the lymphocyte proliferation assay. These peptides were located in four areas containing three to six residues which were distinct for the *M. leprae* product in comparison to that from *M. avium*. Four peptides (p132–151, p206–224 and p267–286), which were the most permissive from each region and recognized by non-contacts with significantly lower frequencies than other subject groups, were identified. From this preliminary result, we conclude that these four peptides were likely to be *M. leprae*-specific.—Authors’ Abstract


This review is a synthesis and analysis of our nine experimental pathology papers on macrophage kinetics in dermal tuberculous lesions produced in rabbits by BCG. It is presented at this time to summarize the macrophage kinetics in both active and essentially healed tuberculous lesions and to suggest that the bacilli frequently multiply and are destroyed in the viable granulation tissue of many small arrested tuberculous lesions. The turnover of mononuclear cells (MN)—which were mostly macrophages with some medium and large lymphocytes—was most rapid in BCG lesions at 2–3 weeks (when tuberculin sensitivity and acquired cellular resistance were at their peaks). At this time, more macrophages entered, more died or left, more remained at the site, and more became activated than before or afterwards. Before this time, the host had no delayed-type hypersensitivity (DTH) and cell-mediated immunity (CMI), so that no antigen-specific enhancement of the inflammatory response occurred. After this time, the bacilli and their antigenic products had decreased, so that the stimuli for cell infiltration and activation were reduced. In “healed” lesions, the MN turnover still occurred, but was decreased. The continuous entry of live non-activated macrophages into the viable parts of tuberculous lesions provides fresh intracellular sites where tubercle bacilli may multiply before they are again inhibited by the DTH and CMI of the host. In tuberculosis, bacillary dormancy of long duration may only be present in caseous necrotic tissue where no live host cells exist.—Author’s Abstract

Severe combined immunodeficiency (SCID) mice were used to analyze the role of NK cells in resistance to *Mycobacterium avium*. The neutralization of IFN-gamma in these animals led to an exacerbation of the infection associated with a reduction in macrophage activation, suggesting a role for NK cells in innate immunity to mycobacteria. In contrast, administration of anti-asialo-GM(1) polyclonal serum or mAb specific for Thy1.2 did not affect mycobacterial growth or macrophage activation despite causing the almost complete abrogation of the natural cytolysis of a tumor cell target. Treatment with anti-asialo-GM(1)-specific serum depleted only two-thirds of the Thy1.2+ spleen cells, and anti-Thy1.2 treatment allowed for the persistence of a small number of cells still exhibiting an NK cell marker recognized by mAb DX5 and able to express IFN-gamma as analyzed by flow cytometry. In *vivo* treatment of B6,SCID mice with anti-NK1.1 mAb again failed to affect resistance to infection and allowed for the persistence of 2–8% of IFN-gamma-producing cells, many of them still expressing the DX5 marker. In *vitro* depletion studies showed that removal of IFN-gamma-expressing cells required the combined action of anti-Thy1.2, anti-Ly49C and DX5 antibodies in the presence of complement. Our data show that resistance to *M. avium* mediated by NK cells is independent of their cytolytic activity, and that there is a marked phenotypic and functional heterogeneity of the NK cell lineage in *vivo* during infection.—Authors’ Abstract


Toll-like receptor (TLR) proteins mediate cellular activation by microbes and microbrial products. To delineate the role of TLR proteins in the development of host immune responses against mycobacteria, wild-type and TLR-deficient mice were infected with nonpathogenic *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). Two weeks after intraperitoneal challenge with BCG, few bacilli were present in the lungs of wild-type and TLR4(−/−) mice, whereas bacterial loads were tenfold higher in the lungs of infected TLR2(−/−) mice. BCG challenge *in vitro* strongly induced proinflammatory cytokine secretion by macrophages from wild-type and TLR4(−/−) mice but not by TLR2(−/−) macrophages. In contrast, intracellular uptake, intracellular bacterial growth, and suppression of intracellular bacterial growth *in vitro* by interferon-gamma (IFN-gamma) (IFN-gamma) were similar in macrophages from all three mouse strains, suggesting that BCG growth in the lungs of TLR2(−/−) mice was a consequence of defective adaptive immunity. Antigenic stimulation of splenocytes from infected wild-type and TLR4(−/−) mice induced T cell proliferation *in vitro*, whereas T cells from TLR2(−/−) mice failed to proliferate. Unexpectedly, activated CD4(+) T cells from both TLR-deficient mouse strains secreted little IFN-gamma *in vitro* compared with control T cells. A role for TLR4 in the control of bacterial growth and IFN-gamma production *in vivo* was observed only when mice were infected with higher numbers of BCG. Thus, TLR2 and TLR4 appear to regulate distinct aspects of the host immune response against BCG.—Authors’ Abstract


*Mycobacterium avium* complex (MAC) is a significant cause of opportunistic infection in patients with acquired immunodeficiency syndrome. Although the major route of entry of MAC is via the gastrointestinal tract, MAC can infect humans through the respiratory tract and eventually encounter alveolar macrophages within the lung. Once in the lung, MAC can potentially interact with surfactant protein A (SP-A), an important component of the pulmonary

The effects of reactional episodes on the cutaneous nerve fibers of leprosy patients was assessed in six patients (three with reversal reactions and three with erythema nodosum leprosum). Cryosections of cutaneous biopsy of reactional lesions taken during the episode and of another sample during the remission period were immunostained with anti-NGFr and anti-PGP 9.5 (indirect immunofluorescence). We found no significant statistical difference in the number of NGFr- and PGP 9.5-positive fibers between the reactional and post-reactional groups. A significant difference was detected between the number of NGFr and PGP 9.5-stained fibers inside of the reactional group of biopsy cryosections but this difference was ascribed to the distinct aspects of the nerve fibers displayed whether stained with anti-NGFr or with anti-PGP 9.5; NGFr-positive branches looked larger and so interpreted as containing more fibers. In addition, a substantial number NGFr-positive fibers were PGP 9.5-negative. No differences in the number of stained fibers among the distinct cutaneous regions examined (epidermis + upper dermis, mid and deep dermis) was detected. In conclusion, the number of PGP- and NGFr-positive fibers were not significantly different in the reactional and post-reactional biopsies in the present study. NGFr-staining of the nerve fibers is different from their PGP-imunoreactivity and the evaluation of the nerve fiber status on an innervated target organ should be carried out choosing markers for both components of nerve fibers (Schwann cells and axons).—Authors’ Abstract


The immunohistochemical identification of neuropeptides (calcitonin gene-related peptide, vasoactive intestinal polypeptide, substance P, alpha-melanocyte stimulating hormone and gamma-melanocyte stimulating hormone) quantification of mast cells and their subsets (tryptase/chymase-immunoreactive mast cells = TCMC and tryptase-immunoreactive mast cells = TMC) were determined in biopsies of six patients with leprosy reactions (three patients with type I reaction and three with type II). Biopsies were compared with those taken from the same body site in the remission stage of the same patient. We found a relative increase of TMC in the inflammatory innate-immune response. Previous work on other pulmonary pathogens including Mycobacterium bovis Bacillus Calmette-Guerin (BCG) suggests that SP-A participates in promoting efficient clearance of these organisms by alveolar macrophages. In the present study, we investigated the role of SP-A in clearance of MAC by cultured rat macrophages. SP-A bound to MAC organisms and enhanced the ingestion of the mycobacteria by macrophages. Infection of macrophages with SP-A-MAC complexes induced the production of nitric oxide (NO) and tumor necrosis factor-alpha. However, intracellular survival of MAC was not altered by preopsonization with SP-A. In addition, inhibitors of inducible NO synthase did not alter MAC clearance. These results suggest that SP-A can bind to and enhance the uptake of MAC by alveolar macrophages, similar to previous findings with BCG and Mycobacterium tuberculosis. However, unlike BCG and other pulmonary pathogens that are cleared effectively in the presence of SP-A via a NO-dependent pathway, macrophage-mediated clearance of MAC is not enhanced by SP-A.—Authors’ Abstract
infiltrate of the reactional biopsies compared to the post-reactional biopsy. Also, the total number of mast cells and the TMC/TCMC ratio in the inflammatory infiltrate was significantly higher than in the intervening dermis of the biopsies of both periods. No significant difference was found regarding neurotide expression in the reactional and post-reactional biopsies. The relative increase of TMC in the reactional infiltrates could implicate this mast cell subset in the reported increase of the immune response in leprosy reactions.—Authors’ Abstract

**Immuo-Pathology (Tuberculosis)**


See Current Literature, Molecular and Genetic Studies, p. 422


In this study we have demonstrated that nitric oxide, the product of the arginine dependent pathway of human mononuclear phagocytes effectively kills the *M. tuberculosis in-vitro*. The release of reactive nitrogen intermediates was triggered by incubation with various proinflammatory cytokines namely IFN gamma, TNF-alpha and IL-1R. We have earlier shown that human mononuclear phagocytes can be induced to release nitric, oxide (NO) radicals which can kill tumour cells. In the present communication, by using colony forming assays we demonstrated that human mononuclear phagocytes can effectively kill *M. tuberculosis* by using a NO dependent pathway. Treatment of mononuclear phagocytes with L-arginine resulted in markedly increased killing activity whereas, by using NGMMA, an analogue of L-arginine, the cidal activity could be brought down to the basal level. These results clearly suggest that cytokines, particularly IFN-gamma, induced NO release and its reactive product with oxygen radical, peroxynitrite, could play an important role in the killing of *M. tuberculosis* by human mononuclear phagocytes. A significant production of interleukin-4 and interleukin-10, by the *ex-vivo* matured, untreated macrophages from the active tuberculosis patients indicate that regulation of cytokine network to encourage in situ/local production of nitric oxide may be useful in the management of pulmonary tuberculosis.—Authors’ Abstract


TNF-deficient mice are highly susceptible to *Mycobacterium tuberculosis* H37Rv infection. Here we asked whether TNF is required for postinfectious immunity in aerosol-infected mice. Chemotherapy for 4 wk commencing 2 wk postinfection reduced CFU to undetectable levels. While wild-type mice had a slight rise in CFU, but controlled infection upon cessation of chemotherapy, TNF-deficient mice developed reactivation of infection with high bacterial loads in lungs, spleen, and liver, which was fatal within 13–18 wk. The increased susceptibility of TNF-deficient mice was accompanied by diminished recruitment and activation of T cells and macrophages into the lung, with defective granuloma formation and reduced inducible NO synthase expression. Reduced chemo-kine production in the lung might explain
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suboptimal recruitment and activation of T cells and uncontrolled infection. Therefore, despite a massive reduction of the mycobacterial load by chemotherapy, TNF-deficient mice were unable to compensate and mount a protective immune response. In conclusion, endogenous TNF is critical to maintain latent tuberculosis infection, and in its absence no specific immunity is generated.—Authors’ Abstract


The identification of immunodominant and universal mycobacterial peptides could be applied to vaccine design and have an employment as diagnostic reagents. In this paper we have investigated the fine specificity, clonal composition and HLA class II restriction of CD4+ T cell clones specific for an immunodominant epitope spanning amino acids 91–110 of the 16-kDa protein of Mycobacterium tuberculosis. Twenty-one of the tested 28 clones had a Th1 profile, while seven clones had a Th0 profile. None of the clones had a Th2 profile. While the TCR AV gene usage of the clones was heterogeneous, a dominant TCR BV2 gene family was used by 18 of the 28 clones. The CDR3 regions of BV2+ T cell clones showed variation in lengths, but a putative common motif R-L/V-G/S-Y/W-E/D was detected in 13 of the 18 clones. Moreover, the last two to three residues of the putative CDR3 loops, encoded by conserved BJ sequences, could also play a role in peptide recognition. Antibody blockade and fine restriction analysis using HLA-DR homozgyous antigen-presenting cells established that 16 of 18 BV2+ peptide-specific clones were DR restricted and two clones were DR-DQ and DR-DP restricted. Additionally, five of the 18 TCRBV2+ clones recognized peptide 91–110 in association with both parental and diverse HLA-DR molecules, indicating their promiscuous recognition pattern. The ability of peptide 91–110 to bind a wide range of HLA-DR molecules, and to stimulate a Th1-type interferon (IFN)-gamma response more readily, encourage the use of this peptide as a subunit vaccine component.—Authors’ Abstract


Mycobacterium tuberculosis interacts with macrophages and epithelial cells in the alveolar space of the lung, where it is able to invade and replicate in both cell types. M. tuberculosis-associated cytotoxicity to these cells has been well documented, but the mechanisms of host cell death are not well understood. We examined the induction of apoptosis and necrosis of human macrophages (U937) and type II alveolar epithelial cells (A549) by virulent (H37Rv) and attenuated (H37Ra) M. tuberculosis strains. Apoptosis was determined by both enzyme-linked immunosorbent assay (ELISA) and TdT-mediated dUTP nick end labelling (TUNEL) assay, whereas necrosis was evaluated by the release of lactate dehydrogenase (LDH). Both virulent and attenuated M. tuberculosis induced apoptosis in macrophages; however, the attenuated strain resulted in significantly more apoptosis than the virulent strain after 5 days of infection. In contrast, cytotoxicity of alveolar cells was the result of necrosis, but not apoptosis. Although infection with M. tuberculosis strains resulted in apoptosis of 14% of the cells on the monolayer, cell death associated with necrosis was observed in 59% of alveolar epithelial cells after 5 days of infection. Infection with M. tuberculosis suppressed apoptosis of alveolar epithelial cells induced by the kinase inhibitor, staurosporine. Because our findings suggest that M. tuberculosis can modulate the apoptotic response of macrophages and epithelial cells, we carried out an apoptosis pathway-specific cDNA microarray analysis of human macrophages and alveolar epithelial cells. Whereas the inhibitors of
apoptosis, bcl-2 and Rb, were upregulated over 2.5-fold in infected (48 hr) alveolar epithelial cells, the proapoptotic genes, bad and bax, were downregulated. The opposite was observed when U937 macrophages were infected with *M. tuberculosis*. Upon infection of alveolar epithelial cells with *M. tuberculosis*, the generation of apoptosis, as determined by the expression of caspase-1, caspase-3 and caspase-10, was inhibited. Inhibition of replication of intracellular bacteria resulted in an increase in apoptosis in both cell types. Our results showed that the differential induction of apoptosis between macrophages and alveolar epithelial cells represents specific strategies of *M. tuberculosis* for survival in the host.—Authors’ Abstract


The ability of peripheral blood mononuclear cells (PBMC) from patients with active tuberculosis to display cytotoxic responses against autologous *Mycobacterium tuberculosis* (Mtbc)-pulsed macrophages was evaluated. Non-MHC restricted cell-dependent lytic activity was observed in ex vivo effector cells from tuberculosis patients and was mediated mainly by CD3(+)-gammadelta TCR(+) T (gammadelta T) cells bearing CD56 and/or CD16 molecules. MHC-restricted and non-MHC restricted cytotoxic T cells (CTL) were differentially expanded upon stimulation with Mtbc in tuberculosis patients and normal controls (N). Class-I restricted CD8(+) CTL and class-II restricted CD4(+) CTL were generated in PPD(+)N and to a lesser extent in PPD(–)N. Mtbc-stimulated effector cells from tuberculosis patients became progressively non-MHC restricted CD4(–)/CD8(–)-gammadelta T cells, while lytic activity of CD4(+) and CD8(+)CTL decreased gradually as the disease became more severe. On the other hand, target cells were lysed by ex vivo cells from tuberculosis patients through the Fas-FasL and perforin pathways. Mtbc-induced CD4(+) CTL from tuberculosis patients and N controls preferentially employed the Fas-FasL mechanism. Mtbc-induced CD8(+) CTL effector cells from patients used the perforin-based mechanism while cells from N controls also used the Fas-FasL pathway. While Mtbc-induced gammadelta CTL from patients and PPD(–)N employed the latter mechanism cells from PPD(+)N individuals also used the perforin pathway. It can be concluded that shifts in the CTL response and the cytolytic mechanisms take place as the pulmonary involvement becomes more severe.—Authors’ Abstract


The possible contribution of NKT cells to resistance to *Mycobacterium tuberculosis* infection remains unclear. In this paper we characterized the Valpha14 NKT cell population following infection with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). BCG infection determined an early expansion of Valpha14 NK T cells in liver, lungs, and spleen, which peaked on day 8 and was sustained until day 30. However, an NK1.1(+) Valpha14 NK T population preferentially producing IFN-gamma predominated at an early stage (day 8), which was substituted by an NK1.1(–) population preferentially producing IL-4 at later stages (day 30). Despite the fact that Valpha14 NKT cell-deficient mice eliminated BCG as did control mice, they had significantly higher numbers of granulomas in liver and lungs. Additionally, while control mice developed organized small granulomas, those in Valpha14 NKT-deficient mice had signs of caseation, large cellular infiltrates, and some multinucleated macrophages, suggesting that Valpha14 NKT cells may actually work as anti-inflammatory cells by limiting excessive lymphocyte influx and tis-
sue pathology. In agreement, we found an increased spontaneous production and mRNA expression of TNF-alpha in liver and lungs of Valpha14 NKT-deficient mice, whose neutralization in vivo by anti-TNF-alpha mAbs consistently reduced the number of granulomas in liver and lungs. Together, our results support a regulatory role for Valpha14 NKT cells in the course of BCG infection through their ability to limit the extent of inflammatory response and point to an important role for this cell subset as a regulator of the balance between protective responses and immunopathology.—Authors’ Abstract


This review provides a discussion on the current information about the response of Mycobacterium tuberculosis to the environment encountered in the macrophage. We focus on the types of genes shown to be upregulated when the pathogen grows in macrophages and discuss the possible roles of these genes in adaptation to the conditions in the eukaryotic cell, in the context of enhancing the survival of the pathogen during infection.—Authors’ Abstract


Mycobacterium tuberculosis is successful as a pathogen because of its ability to persist in an immunocompetent host. This bacterium lives within the macrophage, a cell whose function is the elimination of microbes. Recent advances have improved our understanding of how M. tuberculosis evades two major antimicrobial mechanisms of macrophages: phagolysosome fusion and the production of toxic reactive nitrogen intermediates. M. tuberculosis also modulates antigen presentation to prevent the detection of infected macrophages by CD4(+) T cells.—Authors’ Abstract


Mycobacterium tuberculosis survives in macrophages in the face of acquired CD4(+) T-cell immunity, which controls but does not eliminate the organism. Gamma interferon (IFN-gamma) has a central role in host defenses against M. tuberculosis by activating macrophages and regulating major histocompatibility complex
class II (MHC-II) antigen (Ag) processing. *M. tuberculosis* interferes with IFN-gamma receptor (IFN-gamma R) signaling in macrophages, but the molecules responsible for this inhibition are poorly defined. This study determined that the 19-kDa lipoprotein from *M. tuberculosis* inhibits IFN-gamma-regulated HLA-DR protein and mRNA expression in human macrophages. Inhibition of HLA-DR expression was associated with decreased processing and presentation of soluble protein Ags and *M. tuberculosis* bacilli to MHC-II-restricted T cells. Inhibition of HLA-DR required prolonged exposure to 19-kDa lipoprotein and was blocked with a monoclonal antibody specific for Toll-like receptor 2 (TLR-2). The 19-kDa lipoprotein also inhibited IFN-gamma-induced expression of Fc gamma RI. Thus, *M. tuberculosis*, through 19-kDa lipoprotein activation of TLR-2, inhibits IFN-gamma R signaling in human macrophages, resulting in decreased MHC-II Ag processing and recognition by MHC-II-restricted CD4 T cells. These findings provide a mechanism for *M. tuberculosis* persistence in macrophages.—Authors’ Abstract


Apoptosis is a form of cell death that avoids inflammatory responses. We had previously reported that *Mycobacterium tuberculosis* (Mtb) and Purified Protein Derivative (PPD) induce apoptosis in murine macrophages. The production of TNFalpha and IL-10 in response to Mtb infection modulates apoptosis by controlling nitric oxide production and caspase activation. To further explore the role of macrophage apoptosis in tuberculosis, we studied the capacity of standard antimycobacterial drugs to modulate different events associated with the induction of apoptosis. The B10R murine macrophage line was infected or not with Mtb (5:1 bacteria to macrophage ratio) or exposed to PPD (10 microg/ml), in the presence or absence of varying concentrations (1–20 microg/ml) of anti mycobacterial drugs (isoniazid, rifampin, thiacetzone, streptomycin, and ethambutol). Inhibition of the intracellular growth of *M. tuberculosis* by all drugs studied/correlated with inhibition of permeability transition (PT) alterations; TNFalpha, IL-10, and nitric oxide production, and caspase-1 activation. However, these drugs did not affect PPD-induced apoptosis or its associated events, suggesting that the ability of antimycobacterial drugs to block macrophage apoptosis could be explained by their effects on the metabolic activities of Mtb. All drugs, except isoniazid, at higher concentrations, induced PT alterations in noninfected macrophages in a way that appears to be dependent of calcium, since a calcium chelator prevented it. The results presented herein suggest that the pharmacological manipulation of pathways associated with macrophage apoptosis may affect the intracellular growth of Mtb.—Authors’ Abstract


To induce effector immunity, dendritic cells (DCs) must differentiate into fully mature cells. We show that, after human monocyte-derived DCs were infected with virulent *Mycobacterium tuberculosis*, up-regulation of cellular-surface maturation markers was minimal and reversible. In the presence of a potent stimulus for maturation (tumor necrosis factor [TNF]-alpha, interleukin [IL]-1beta, and prostaglandin E2 [PGE2]), *M. tuberculosis* inhibited phenotypic DC maturation. *M. tuberculosis*-infected DCs had an impaired ability to induce allogeneic lymphoproliferation and activated autologous memory CD4+ and CD8+ T cells optimally only in the presence of TNF-alpha, IL-1beta, and PGE2. Thus, virulent *M. tuberculosis* inhibits phenotypic and functional maturation of human monocyte-derived DCs. This mechanism, which has been described elsewhere for various viruses and for the virulent mycobacte-
rium *M. leprae*, may be a novel mechanism that this pathogen uses to evade the host’s immune response.—Authors’ Abstract


CD8(+) T cells play a central role in immune protection against infection with *Mycobacterium tuberculosis*. One of the target epitopes for anti-*M. tuberculosis* directed CD8(+) T cells is the HLA-A2-restricted 19-kDa lipoprotein peptide VLTDGNPPEV. T cell clones directed against this epitope recognized not only the nominal peptide ligand, but also a closely related peptide (VPTDPNPPEV) from the HIV envelope gp120 (HIV(env) gp120) protein characterized by IFN-gamma release. This cross-reactivity was confirmed in *ex vivo* in *M. tuberculosis* 19-kDa tetramer-sorted T cells from patients with tuberculosis and in HIV gp120 tetramer-reactive T cells sorted from HIV(+) patients. *M. tuberculosis* 19-kDa antigen-reactive T cells were present in HLA-A2(+) patients (10/10) with HIV infection with no evidence of *M. tuberculosis* infection, but they are absent in peripheral blood lymphocytes from healthy HLA-A2(+) individuals (10/10). *M. tuberculosis* 19-kDa antigen-reactive T cells were elevated in acute pulmonary tuberculosis, declined with response to therapy (7/10 patients) and resided in the terminally differentiated CD8(+) T cell subset. CD8(+) cross-reactive T cells recognizing HIV(env) or *M. tuberculosis* 19-kDa antigens may contribute to pathogenesis in individuals co-infected with both pathogens and may also present a marker for active tuberculosis.—Authors’ Abstract


Th1 immune response is essential in the protection against mycobacterial intracellular pathogens. Lipoproteins trigger both humoral and cellular immune responses and may be candidate protective antigens. We studied in BALB/c mice the immunogenicity and the protection offered by the recombinant 27-kDa *Mycobacterium tuberculosis* lipoprotein and the corresponding DNA vaccine. Immunization with the 27-kDa antigen resulted in high titers of immunoglobulin G1 (IgG1) and IgG2a with a typical Th1 profile and a strong delayed hypersensitivity response. A strong proliferation response was observed in splenocytes, and significant nitric oxide production and gamma interferon secretion but not interleukin 10 secretion were measured. Based on these criteria, the 27-kDa antigen induced a typical Th1-type immune response thought to be necessary for protection. Surprisingly, in 27-kDa-vaccinated mice (protein or DNA vaccines) challenged by *M. tuberculosis* H37Rv or BCG strains, there was a significant increase in the numbers of CFU in the spleen compared to that for control groups. Furthermore, the protection provided by BCG or other mycobacterial antigens was completely abolished once the 27-kDa antigen was added to the vaccine preparations. This study indicates that the 27-kDa antigen has an adverse effect on the protection afforded by recognized vaccines. We are currently studying how the 27-kDa antigen modulates the mouse immune response.—Authors’ Abstract


PURPOSE OF REVIEW: Cytokines have been implicated in the protective immunity, pathophysiology and development of tuberculosis. Most people who become infected with *Mycobacterium tuberculosis* mount an effective protective immune re-
response, but 5–10% develop disease. Active pulmonary tuberculosis can be considered to reflect an ineffective immune response against mycobacterial infection. A better understanding of how cytokine production contributes to immunity and pathology would aid the development of new vaccines and therapeutic strategies. RECENT FINDINGS: At the time of diagnosis, production of *M. tuberculosis* or mycobacterial antigen-induced interferon-gamma by peripheral blood mononuclear cells from tuberculosis patients is usually depressed, compared with that of healthy control subjects, whereas cytokine production at the site of disease is elevated. In most patients, depressed interferon-gamma production by peripheral blood mononuclear cells seems to be a transient response because it is significantly increased in most active tuberculosis patients during and following successful antituberculous therapy. However, some patients remain anergic in vivo and in vitro after chemotherapy, and the underlying biochemical mechanisms for T cell anergy in modulating protection or pathology in tuberculosis needs further clarification. Among the cytokines contributing to protective immunity, interleukins 12 and 18, and tumour necrosis factor-alpha are important, the basis of recent studies with tuberculosis patients. SUMMARY: A more complete understanding of cytokine dynamics in individual cells in active pulmonary tuberculosis patients will provide further knowledge about immunopathogenesis and protective immunity in human tuberculosis. This should ultimately enhance development of preventive and therapeutic strategies against this enormously successful intracellular pathogen. —Authors’ Abstract


*Mycobacterium tuberculosis* produces a variety of molecules capable of activating Toll-like receptors, a family of pattern recognition receptors expressed by macrophages and a variety of other cells. To determine whether Toll-like receptor 4 (TLR4) was critical in resistance to *M. tuberculosis* infection, we compared the morbidity and mortality of TLR4-defective C3H/HeJ mice to those of TLR4-sufficient C3H mouse substrains. TLR4-defective C3H/HeJ mice and TLR4-sufficient C3H/HeSnJ, C3HeB/FfJ, and C3H/HeOuJ mice were infected by the aerosol route with *M. tuberculosis*. TLR4-defective C3H/HeJ mice had levels of cytokines in their bronchoalveolar lavage fluids and in vitro mycobacterial antigen-specific recall responses similar to those of other C3H mouse substrains. In addition, bacterial replication and long-term survival of mice following infection appeared to be independent of TLR4. Interestingly, C3HeB/FfJ mice were significantly more susceptible to *M. tuberculosis* infection, indicating that genetic heterogeneity among inbred C3H mouse substrains modifies resistance to infection. Therefore, cautious interpretation is required when the C3H/HeJ strain is used as a model of a TLR4-defective mouse strain, as there are significant allelic differences between C3H/HeJ and other C3H mouse substrains in response to *M. tuberculosis* infection. With this caveat, our data indicate that TLR4 may not be required for optimal immunity of mice to *M. tuberculosis*. —Authors’ Abstract


Elevated levels of circulating adhesion molecules in patients with active pulmonary tuberculosis MUKAE H, ASHITANI J-I, TOKOJIMA M, IHI T, KOHNO S, MATSUKURA S. Respirology 2003; 8: 326–331 OBJECTIVE: Recent studies have indicated the importance of cell adhesion molecules in the pathogenesis of various inflammatory lung diseases. Our study was designed to determine whether five soluble adhesion molecules including soluble L-, E- and P-selectin (sL-, sE- and sP-selectin), intercellular adhesion molecule-1 (sICAM-1), and vascular cell adhesion molecule-1 (sVCAM-1) in serum reflect the severity of
active pulmonary tuberculosis (TB), and whether there is a distinct profile of these soluble molecules in this disease. METHODOLOGY: Using enzyme-linked immunosorbent assays, we measured the serum levels of these five soluble adhesion molecules in 31 patients with active TB and 11 healthy volunteers. RESULTS: Serum levels of sE-selectin, sP-selectin and sICAM-1, but not sL-selectin or sVCAM-1, were significantly higher in patients with active TB than in the control subjects (p <0.001, each). Significant correlations were detected only between serum levels of sE-selectin and sP-selectin, sE-selectin and sICAM-1, and sP-selectin and sICAM-1. There was a significant correlation between the Gaffky scale result (a scale assessing the number of mycobacteria bacilli present) and all of the above adhesion molecules, except for sL-selectin. Serum levels of sE-selectin, sL-selectin and sICAM-1 also correlated with the CXR radiological score. Higher levels of sL-selectin and sICAM-1 were detected in the serum of patients with radiological cavity formation compared to those without. The ESR, C-reactive protein and circulating neutrophil counts all correlated significantly with sE-selectin, sP-selectin, sICAM-1 and sVCAM-1. CONCLUSION: The results suggest that there is a distinct profile of soluble adhesion molecules in active pulmonary TB and that sE-selectin, sP-selectin, and especially sICAM-1 appear to be the most sensitive clinical measures of disease severity.—Authors’ Abstract

Microbiology


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Mycobacterial interspersed repetitive units (MIRU) comprise short tandem repeat structures found at multiple loci throughout the Mycobacterium tuberculosis genome and have been used for typing these pathogens. We have identified MIRU at 18 conserved loci throughout the common portions of the Mycobacterium avium subspecies paratuberculosis (MAP) and M. avium subspecies avium (MAA) genomes. Six of these loci were found to differ between MAA and MAP in the number of tandem repeat motifs occurring at each MIRU locus. Locus specific PCR at 4 of these loci segregated MAP into two major groups, which could be differentiated from ovine-pigmented strains of MAP and the MAP vaccine strain 316F. The same PCR differentiated MAA into five MIRU profiles. PCR at either MIRU locus 1 or MIRU locus 4 distinguished MAP from all other Mycobacterium intracellulare. MIRU typing may provide an additional simple and rapid procedure for the differentiation between MAP and other M. avium complex (MAC) tested. PCR at both loci 1 and 4 also distinguished MAP from Mycobacterium intracellulare. MIRU typing may provide an additional simple and rapid procedure for the differentiation between MAP and other MAC.—Authors’ Abstract


Mycobacteria, like many prokaryotes, have a peptidoglycan with peptides composed of L-alanine (or glycine), D-iso-glutamine, meso-diaminopimelate, and
D-alanine. We sought to study mycobacterial peptidoglycan biosynthesis by constructing diaminopimelate (DAP) auxotrophs of Mycobacterium smegmatis and then isolating spontaneous mutants of these auxotrophs that can grow in the absence of DAP. Here we report the isolation and characterization of seven classes of spontaneous M. smegmatis mutants with extragenic mutations that can suppress the DAP requirement of DAP auxotrophs.—Authors’ Abstract


The susceptibility of representative strains of Mycobacterium avium, Mycobacterium intracellulare, and Mycobacterium scrofulaceum (the MAIS group) to chlorine was studied to identify factors related to culture conditions and growth phase that influenced susceptibility. M. avium and M. intracellulare strains were more resistant to chlorine than were strains of M. scrofulaceum. Transparent and unpigmented colony variants were more resistant to chlorine than were their isogenic opaque and pigmented variants (respectively). Depending on growth stage and growth rate, MAIS strains differed in their chlorine susceptibilities. Cells from strains of all three species growing in early log phase at the highest growth rates were more susceptible than cells in log and stationary phase. Rapidly growing cells were more susceptible to chlorine than slowly growing cells. The chlorine susceptibility of M. avium cells grown at 30 degrees C was increased when cells were exposed to chlorine at 40 degrees C compared to susceptibility after exposure at 30 degrees C. Cells of M. avium grown in 6% oxygen were significantly more chlorine susceptible than cells grown in air. Chlorine-resistant MAIS strains were more hydrophobic and resistant to Tween 80, para-nitrobenzoate, hydroxylamine, and nitrite than were the chlorine-sensitive strains.—Author’s Abstract


Mycobacterium bovis is the causative agent of tuberculosis in a range of animal species and man, with worldwide annual losses to agriculture of $3 billion. The human burden of tuberculosis caused by the bovine tubercle bacillus is still largely unknown. M. bovis was also the progenitor for the M. bovis bacillus Calmette-Guerin vaccine strain, the most widely used human vaccine. Here we describe the 4,345,492-bp genome sequence of M. bovis AF2122/97 and its comparison with the genomes of Mycobacterium tuberculosis and Mycobacterium leprae. Strikingly, the genome sequence of M. bovis is >99.95% identical to that of M. tuberculosis, but deletion of genetic information has led to a reduced genome size. Comparison with M. leprae reveals a number of common gene losses, suggesting the removal of functional redundancy. Cell wall components and secreted proteins show the greatest variation, indicating their potential role in host-bacillus interactions or immune evasion. Furthermore, there are no genes unique to M. bovis, implying that differential gene expression may be the key to the host tropisms of human and bovine bacilli. The genome sequence therefore offers major insight on the evolution, host preference, and pathobiology of M. bovis.—Authors’ Abstract

Although *Mycobacterium kansasii* has emerged as an important pathogen frequently encountered in immunocompromised patients, little is known about the mechanisms of *M. kansasii* pathogenicity. Lipoarabinomannan (LAM), a major mycobacterial cell wall lipoglycan, is an important virulence factor for many mycobacteria, as it modulates the host immune response. Therefore, the detailed structures of the of *M. kansasii* LAM (KanLAM), as well as of its biosynthetic precursor lipomannan (KanLM), were determined in a clinical strain isolated from a human immunodeficiency virus-positive patient. Structural analyses revealed that these lipoglycans possess important differences as compared with those from other mycobacterial species. KanLAM carries a mannooligosaccharide cap but is devoid of the inositol phosphate cap present in *Mycobacterium smegmatis*. Characterization of the mannan core of KanLM and KanLAM demonstrated the following occurrences: 1) alpha1,2-oligo-mannopyranosyl side chains, contrasting with the single mannopyranosyl residues substituting the mannan core in all the other structures reported so far; and 2) 5-methylthiopentose residues that were described to substitute the arabinan moiety from *Mycobacterium tuberculosis* LAM. With respect to the arabinan domain of KanLAM, succinyl groups were found to substitute the C-3 position on 5-arabinofuranosyl residues, reported to be linked to the C-2 of the 3,5-arabinofuranose in *Mycobacterium bovis* bacillus calmette-guerin LAM. Because *M. kansasii* has been reported to induce apoptosis, we examined the possibility of the *M. kansasii* lipoglycans to induce apoptosis of THP-1 cells. Our results indicate that, in contrast to KanLAM, KanLM was a potent apoptosis-inducing factor. This work underlines the diversity of LAM structures among various pathogenic mycobacterial species and also provides evidence of LM being a potential virulence factor in *M. kansasii* infections by inducing apoptosis.—Authors’ Abstract

**Microbiology (Leprosy)**


*Mycobacterium leprae* cells (strain Thai-53) harvested from infected mouse foot pads were examined by electron microscopy using the freeze-substitution technique. The population of *M. leprae* cells from the infected tissue consisted of a large number of degraded cells and a few normal cells. These thin sectioned cell profiles could be categorized into four groups depending on the alteration of the membrane structures, and the degradation process is considered to occur in stages, namely from stages 1 to 3. These are the normal cells with an asymmetrical membrane, a seemingly normal cell but with a symmetrical membrane (stage 1), a cell possessing contracted and highly concentrated cytoplasm with a membrane (stage 2), and a cell that has lost its membrane (stage 3). The peptidoglycan layer was found to remain intact in these cell groups.—Authors’ Abstract


To determine the best molecular method for diagnosing leprosy, two sets of *Mycobacterium leprae*-specific primers were compared. Fresh biopsies and slit skin smear samples were obtained from 67 leprosy patients and examined by touchdown (TD) PCR using primers amplifying either a 129-bp fragment of the RLEP repetitive sequence or a 360-bp fragment of the 18-kDa protein gene of *M. leprae*. Seventeen of 30 (56.7%) biopsy specimens and four of
37 (10.8%) slit skin smear specimens were positive using the primer for the 18-kDa protein gene, whereas 24 of 30 (80%) biopsy and 27 of 37 (73%) slit skin smear samples showed detectable PCR products in the RLEP repetitive sequence. Twenty-one of 31 cases (67.7%) with a bacterial index of zero were PCR positive for the primer RLEP repetitive sequence. These results demonstrate that detection of \textit{M. leprae} using PCR with primers to a RLEP sequence is more sensitive and specific than PCR with the 18-kDa protein gene primers and also slit smears with acid fast staining. PCR of RLEP repetitive sequences is therefore a useful means of detecting \textit{M. leprae} DNA even when it is present at very low levels.—Authors’ Abstract

**Microbiology (Tuberculosis)**


In a prospective study conducted in a diagnostic laboratory in Mexico City, luciferase reporter mycobacteriophages (LRPs) were evaluated for their utility and performance in identification and antibiotic-susceptibility testing of \textit{Mycobacterium tuberculosis} complex (MTC) isolates from MGIT-960 cultures. Eighty-four consecutive MGIT cultures recovered from 54 patients were included in this study. The LRPs confirmed mycobacterial growth in 79 (94%) of 84 MGIT cultures. Failure to confirm growth was due to low inoculum (N = 1) or growth with non-tuberculous mycobacteria (N = 4). The median time to confirmation of MGIT cultures was 1 day (range 1–55). Confirmed cultures were identified with p-nitro-alpha-acetylamino-beta-hydroxypropiophenone (NAP), a selective inhibitor of MTC species, and results obtained with LRPs were compared with those obtained by BACTEC-460. The sensitivity and specificity of the LRP NAP test were respectively 97 and 100%, and the median turnaround time for identification was 3 days with both methods. The accuracy and speed of the LRPs for susceptibility testing with rifampicin, streptomycin, isoniazid and ethambutol were compared with BACTEC-460 and discrepant results were tested by the conventional agar proportion method.

In total, 72 MTC cultures were tested. The overall agreement between the LRPs and BACTEC-460 was 98.6%. Four isolates (5.6%) were falsely identified as ethambutol-resistant. The median turnaround time for susceptibility testing was 3 days (range 3–57) with the LRPs and 9 days (range 7–29) with BACTEC-460. LRPs offer an accurate and rapid approach for identification and susceptibility testing of \textit{M. tuberculosis} from MGIT-960 cultures.—Authors’ Abstract


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Long-term survival of nonreplicating \textit{Mycobacterium tuberculosis} (Mtb) is ensured by the coordinated shutdown of active metabolism through a broad transcriptional pro-
gram called the stringent response. In Mtb, this response is initiated by the enzymatic action of RelMtb and deletion of relMtb produces a strain (H37RvDeltarelMtb) severely compromised in the maintenance of long-term viability. Although aerosol inoculation of mice with H37RvDeltarelMtb results in normal initial bacterial growth and containment, the ability of this strain to sustain chronic infection is severely impaired. Significant histopathologic differences were noted in lungs and spleens of mice infected with H37RvDeltarelMtb compared with controls throughout the course of the infection. Microarray analysis revealed that H37RvDeltarelMtb suffers from a generalized alteration of the transcriptional apparatus, as well as specific changes in the expression of virulence factors, cell-wall biosynthetic enzymes, heat shock proteins, and secreted antigens that may alter immune recognition of the recombinant organism. Thus, RelMtb is critical for the successful establishment of persistent infection in mice by altering the expression of antigenic and enzymatic factors that may contribute to successful latent infection.—Authors’ Abstract


Previous work has shown that the divergently transcribed Mycobacterium tuberculosis genes acr (hspX, Rv2031c) and acg (Rv2032) are induced under conditions of shallow standing culture and low oxygen and intracellularly within macrophages. We used a combination of computational and experimental methods to identify promoters for eight additional genes that are regulated in a similar manner and that comprise an acr-coregulated promoter (ACP) family. Transcriptional regulation of these ACP family members was evaluated by using a plasmid-based promoter-green fluorescent protein fusion system and flow cytometry. All promoters showed increased expression in shallow standing versus shaking cultures, in low- versus high-oxygen conditions, and intracellularly within macrophages versus extracellularly in tissue culture medium. However, there were quantitative differences in expression among promoters and among conditions for each promoter. A conserved 18-bp palindromic sequence motif was identified in all ACPs by Gibbs sampling-based computational analyses. Two such motifs overlap regions in the acr and acg promoters that were previously shown to be required for their expression. In addition, we found that 5% carbon dioxide was required for growth of Mycobacterium bovis BCG under microaerophilic (1.3% O2) culture conditions and fully prevented the growth cessation typically associated with rapid removal of oxygen. These findings are likely to be relevant to the in vivo environment and will contribute to our understanding of the pathogenesis of tuberculosis infection.—Authors’ Abstract


To confirm that Mycobacterium tuberculosis chaperonin 10 (Cpn10) is secreted outside the live bacillus, infected macrophages were examined by electron microscopy. This revealed that the mycobacterial protein accumulates both in the wall of the bacterium and in the matrix of the phagosomes in which ingested mycobacteria survive within infected macrophages. To understand the structural implications underlying this secretion, a structural study of M. tuberculosis Cpn10 was performed under conditions that are generally believed to mimic the membrane environment. It was found that in buffer-organic solvent mixtures, the mycobacterial protein forms two
main species, namely, a partially helical monomer that prevails in dilute solutions at room temperature and a dimer that folds into a beta-sheet-dominated structure and prevails in either concentrated protein solutions at room temperature or in dilute solutions at low temperature. A partially helical monomer was also found and was completely associated with negatively charged detergents in a micelle-bound state. Remarkably, zwitterionic lipids had no effect on the protein structure. By using N- and C-truncated forms of the protein, the C- and N-terminal sequences were identified as possessing an amphiphilic helical character and as selectively associating with acidic detergent micelles. When the study was extended to other chaperonins, it was found that human Cpn10 is also monomeric and partially helical in dilute organic solvent-buffer mixtures. In contrast, *Escherichia coli* Cpn10 is mostly dimeric and predominately beta-sheet in both dilute and concentrated solutions. Interestingly, human Cpn10 also crosses biological membranes, whereas the *E. coli* homologue is strictly cytosolic. These results suggest that dissociation to partially helical monomers and interaction with acidic lipids may be two important steps in the mechanism of secretion of *M. tuberculosis* Cpn10 to the external environment.—Authors’ Abstract


*Mycobacterium tuberculosis* infects one-third of the world’s population and causes two million deaths annually. The unusually low permeability of its cell wall contributes to the ability of *M. tuberculosis* to grow within host macrophages, a property required for pathogenesis of infection. *Mycobacterium marinum* is an established model for discovering genes involved in mycobacterial infection. *Mycobacterium marinum* mutants with transposon insertions in the beta-ketoacyl-acyl carrier protein synthase B gene (kasB) grew poorly in macrophages, although growth *in vitro* was unaffected. Detailed analyses by thin-layer chromatography, nuclear magnetic resonance (NMR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, infrared spectroscopy, and chemical degradations showed that the kasB mutants synthesize mycolic acids that are 2–4 carbons shorter than wild type; the defect was localized to the proximal portion of the meromycolate chain. In addition, these mutants showed a significant (approximately 30%) reduction in the abundance of keto-mycolates, with a slight compensatory increase of both alpha- and methoxy-mycolates. Despite these small changes in mycolate length and composition, the kasB mutants exhibited strikingly altered cell wall permeability, leading to a marked increase in susceptibility to lipophilic antibiotics and the host antimicrobial molecules defensin and lysozyme. The abnormalities of the kasB mutants were fully complemented by expressing *M. tuberculosis* kasB, but not by the closely related gene kasA. These studies identify kasB as a novel target for therapeutic intervention in mycobacterial diseases.—Authors’ Abstract

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SETTING: Tuberculosis Research Centre, Chennai, India. OBJECTIVE: To rapidly identify multidrug-resistant *Mycobacterium tuberculosis* using phenotypic and genotypic methods. DESIGN: Two genotypic assays, DNA sequencing and polymerase chain reaction single strand conformation polymorphism (PCR-SSCP), and one phenotypic assay, phage amplified biological assay (PhaB) were standardised in-house and performed on coded 101 rifampicin-resistant and 100 rifampicin-sensitive *M. tuberculosis* clinical isolates for the identification of rifampicin resistance. RESULTS AND CONCLUSION: The results obtained using the three assays were compared with those from the conventional indirect sensitivity test. The sensitivities and specificities of DNA sequencing, PCR-SSCP and PhaB were 97% and 100%, 76% and 100%, and 97% and 84%, respectively. DNA sequencing was found to be more sensitive and specific than the other tests.—Authors’ Abstract


AIMS: Despite its long history, the acid fast smear remains unstandardised. Technical variations in both the preparation of clinical material and subsequent staining mean that smear sensitivity relative to culture may vary from 50% to over 80%. This study assessed the sensitivity of acid fast microscopy at each of five stages of sample preparation and by both commonly used staining methods. METHODS: Sputum samples thought for varying reasons to be highly likely to be culture positive were used to prepare a series of smears in which the effects of digestion (liquefaction), concentration (centrifugation), and decontamination (sodium hydroxide) could be assessed, together with a comparison of staining by the auramine/phenol and Ziehl-Neelsen techniques. RESULTS: The most effective method for the demonstration of acid fast organisms in sputum was found to be an auramine phenol stain applied to a liquefied, concentrated sample and examined before the decontamination process. CONCLUSIONS: The auramine phenol stain applied to a liquefied, concentrated sample and examined before the decontamination process is the most effective method for the demonstration of acid fast organisms in sputum.—Authors’ Abstract
Experimental Infections


DNA-based vaccines generate potent cellular immunity as well as humoral immunity. It seems evident that cytokines play a crucial role in generation of effector T cell subsets and in determining the magnitude of the response by DNA vaccines. In this study, we compared the effects of several TH1 cytokine genes as adjuvant in DNA vaccination using mycobacterial Hsp65 as a model antigen. Our results demonstrated that although the overall immune response to Hsp65 was enhanced by co-injection of Hsp65 DNA with cytokine genes, each cytokine gene was shown to affect different immune response elements. Co-injection of Hsp65 DNA with IL-12 or GM-CSF led to an increase in IFN-gamma production and represented potent protections against *Mycobacterium tuberculosis* challenge, while that with Eta-1, IL-12 or IL-18 gene led to an elevated IgG2a/IgG1 ratio. Interestingly, co-administration of Flt3L gene was shown to enhance the Ag-specific CTL response. These results show that the direction and magnitude of immune response in DNA vaccination against Hsp65 of *M. tuberculosis* could be modulated in different ways by co-injection of an appropriate cytokine gene as adjuvant.—Authors’ Abstract


Lipids and glycolipid molecules derived from *Mycobacterium tuberculosis* can be presented to T cells by CD1 cell-surface molecules in humans. These lipid-specific T cells are cytolytic, secrete pro-inflammatory cytokines and have bactericidal activity. Here, we describe studies in which lipids from *M. tuberculosis* were incorporated into liposomes with adjuvant and tested as vaccines in a guinea pig aerosol tuberculosis challenge model. Animals vaccinated with mycobacterial lipids showed reduced bacterial burdens in the lung and spleen at 4 weeks after infection. In addition, the lungs of lipid-vaccinated animals also had significantly less pathology, with granulomatous lesions being smaller and more lymphocytic. In contrast, animals receiving only vehicle control immunizations had granulomatous lesions that were larger and often contained caseous necrotic centers. Quantification of histopathology by morphometric analysis revealed that the overall percentage of lung occupied by diseased tissue was significantly smaller in lipid-vaccinated animals as compared to vehicle control animals. In addition, the mean area of individual granulomatous lesions was found to be significantly smaller in both lipid- and bacillus Calmette-Guerin-vaccinated guinea pigs. These data support an important role for lipid antigens in the immune response to *M. tuberculosis* infection, potentially through the generation of CD1-restricted T cells. Immunogenic lipids thus represent a novel class of antigens that might be included to enhance the protective effects of subunit vaccine formulations.—Authors’ Abstract


The influx of macrophages into the lungs is the major component of the granulomatous response to infection with *Mycobacterium tuberculosis*. In this investigation we used flow cytometric analysis to define macrophage populations entering the airways and lung tissues of infected mice. We
demonstrate that by the judicious use of cell surface markers, especially CD11b and CD11c, several cell populations can be distinguished, allowing cell sorting and morphological definition. Primary populations of CD11b(−)/CD11c(+/high) were defined as alveolar macrophages, CD11b(high)/CD11c(+/high) as dendritic cells, and CD11b(+/mid)/CD11c(+/mid) as small macrophages or monocytes, and changes in the activation phenotype of these populations were followed over the early course of the infection. In further studies, these cell populations were compared with cells harvested during the chronic stage of the disease. During the chronic stage of infection, Ag-presenting class II molecules and activation markers were poorly expressed on dendritic, small macrophage, and monocyte cell populations, which may have important implications for the breakdown of the lesions during reactivation disease. This analytical approach may facilitate the further characterization of macrophage populations entering into the lung tissues and their relative contributions to host resistance to tuberculosis infection.—Authors’ Abstract


The prevention of Mycobacterium tuberculosis (M. tuberculosis) reactivation would greatly reduce the incidence of the disease, particularly among the elderly. Here, we evaluated the efficacy of DNA vaccine in combination with a conventional TB chemotherapy on the prevention of M. tuberculosis reactivation. Mice were treated with isoniazid and pyrazinamide for 3 months from 4 weeks after aerosol infection with M. tuberculosis H37Rv. During this period of chemotherapy, DNA immunization was performed three times monthly with an antigen 85A (Ag85A) DNA or an IL-12 mutant (IL-12N220L) DNA, which is known to lead to a reduction in the secretion of the p40 subunit, but not of a bioactive IL-12p70. The reactivation of M. tuberculosis was dramatically reduced in mice treated with either Ag85A DNA (p <0.01) or IL-12N220L DNA (p <0.05) in combination with chemotherapy, compared with control mice receiving only chemotherapy. Ag85A DNA vaccine showed higher IFN-gamma responses to Ag85A protein, but a lower response to culture filtrate than IL-12N220L DNA vaccine. In addition, Ag85A DNA vaccine prevented the reactivation of M. tuberculosis more efficiently than IL-12N220L DNA vaccine, indicating
that Ag85A-specific IFN-gamma response might correlate with M. tuberculosis control. This study suggests that immunotherapy using Ag85A or IL-12N220L DNA vaccine combined with conventional chemotherapy might be effective clinically for the prevention of tuberculosis reactivation and may offer a more effective cure for humans than chemotherapy alone.—Authors’ Abstract


Lipoarabinomannan (LAM) is a major structural surface component of mycobacteria. Arabinomannan (AM) oligosaccharides derived from LAM of Mycobacterium tuberculosis H37Rv were isolated and covalently conjugated to tetanus toxoid (TT) or to short-term culture filtrate proteins (antigen 85B (Ag85B) or a 75kDa protein) from M. tuberculosis strain Harlingen. The different AM oligosaccharide (AMOs)-protein conjugate vaccine candidates proved to be highly immunogenic, inducing boosterable IgG responses against the AMOs portion of the conjugates in rabbits and guinea-pigs. Proliferation of T-cells from C57BL/6 mice immunized with the conjugates was seen upon in vitro stimulation with PPD. In C57BL/6 mice subcutaneous immunization with the AMOs-antigen 85B conjugate in alum provided significant protection compared to sham (alum only) immunized mice (p <0.021) as estimated by long term survival against intravenous challenge with 10(5) M. tuberculosis H37Rv. Subcutaneous immunization followed by nasal boost with an AMOs-TT conjugate in Eurocine L3 adjuvant provided high (p <0.025) protection as determined by long term survival after intranasal challenge with 10(5) virulent M. tuberculosis strain Harlingen. This level of protection was comparable to that obtained with the conventional live attenuated BCG vaccine. In guineapigs, immunization with AMOs-Ag85B in Eurocine L3 adjuvant followed by aero- genic challenge with M. tuberculosis H37Rv resulted in increased survival and reduced pathology in lungs and spleens relative to non-immunized animals.—Authors’ Abstract


Immune responses of lymphocyte populations during early phases of mycobacterial infection and reinfection have not been well characterized in humans. A non-human primate model of Mycobacterium bovis bacille Calmette-Guerin (BCG) infection was employed to characterize optimally the immune responses of mycobacteria-specific T cells. Primary BCG infection induced biphasic immune responses, characterized by initial lymphocytopenia and subsequent expansion of CD4+, CD8+ and gammadelta T cell populations in the blood, lymph nodes and the pulmonary compartment. The potency of detectable T cell immune responses appears to be influenced by the timing and route of infection as well as challenge doses of BCG organisms. Systemic BCG infection introduced by intravenous challenge induced a dose-dependent expansion of circulating CD4+, CD8+ and gammadelta T cells whereas, in the pulmonary compartment, the systemic infection resulted in a predominant increase in numbers of gammadelta T cells. In contrast, pulmonary exposure to BCG through the bronchial route induced detectable expansions of CD4+, CD8+ and gammadelta T cell populations in only the lung but not in the blood. A rapid recall expansion of these T cell populations was seen in the macaques reinfected intravenously and bronchially with BCG. The expanded alpha/beta and gammadelta T cell populations exhibited their antigen specificity for mycobacterial peptides and non-peptide phospholigands, respectively. Finally, the major expansion of T cells was associated with a resolution of active BCG infection and reinfection. The patterns and kinetics of CD4+, CD8+ and gammadelta T cell immune responses during BCG infection might con-
tribute to characterizing immune protection against tuberculosis and testing new tuberculosis vaccines in primates.—Authors’ Abstract


The distribution and expression of CD40, its ligand CD40L (154) and related cytokines interleukin-12 (IL-12), tumour necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma) and transforming growth factor-beta1 (TGF-beta1) were studied in the lungs of B6D2F1 hybrid mice during slowly progressive primary tuberculosis (TB) by immunohistochemistry. CD40 and CD40L are implicated in cell-mediated immunity (CMI) causing activation or apoptosis of infected cells. The phenomenon of apoptosis is associated with *Mycobacterium tuberculosis* survival. In this study, using frozen lung sections (N = 33), our results showed increased CD40, IL-12 and TGF-beta expression in macrophages with progression of disease. High percentages of mycobacterial antigens (M.Ags), CD40L and IFN-gamma expression were maintained throughout infection, and TNF-alpha-expressing cells were decreased. In lymphocytes, the percentage of IFN-gamma-positive cells was increased, but CD40L and IL-12 were maintained with the progression of disease. M.Ags, CD40 and CD40L were expressed in the same areas of the lesions. We conclude that changes in the expression of CD40-CD40L and cytokines associated with *M. tuberculosis* infection favour the hypothesis that *M. tuberculosis* causes resistance of host cells to apoptosis causing perpetuation of infection.—Authors’ Abstract

**Epidemiology and Prevention**


A descriptive epidemiologic study on the detection of new leprosy cases was conducted in Sao Luis, Maranhao, Brazil, from 1993 to 1998. A database was created for the purpose, covering 2796 reported cases. General detection rates were calculated, as well as specific rates by gender, clinical type, and age group. Linear, exponential, geometric, and log adjustment models were performed to analyze time trends in the disease. An increase in detection was observed, involving mostly female and paucibacillary cases, mainly of tuberculoid leprosy. The increase in detection was most evident in the 15 to 19 year-old population. The percentage of detection under 15 indicated the need for active case search in this group.—Authors’ Abstract


Leprosy is an infectious disease for which humans are considered the only source of infection. The major hindrance in leprosy control and thus in reaching the elimination goal is that numerous leprosy cases remain undetected for a long time. Many of these patients are a continuous source of infection and, hence perpetuate transmission. The goal of the World Health Organization (WHO) is to eliminate leprosy as a public problem by the year 2000; that is, to reach as a global prevalence of <1 per 10,000 people. The epidemiological data generated routinely by health services are greatly influenced by their policies and activities. The data do not, however necessarily reflect the true situation in the field. Information on the magnitude of the leprosy problem in any one area is important for the health services with regard to their planning, monitoring
and evaluation of leprosy control activities. Our studies have suggested that the high prevalence of antibodies in children may be indicative of the active transmission of *M. leprae* in their surroundings. The prevalence of these antibodies may also be important for leprosy control programs in order to detect new patients as early as possible and in an effective and sustainable manner. Based on PCR data, it seems that the environment also plays an important role in the transmission of leprosy in endemic areas. The results of our study show that contact with a leprosy patient is the major determinant in the incidence of leprosy and that this concept shows similarities with the “stone-in-the-pond” principle of tuberculosis transmission in concentric circle around patients.—Author’s Abstract

**Kumar, A., Girdhar, A., Girdhar, B. K.**


During May 2000–June 2001, a survey was carried out in the urban areas of Agra, India, to evaluate the prevalence of leprosy in the area. A total of 60,179 persons from more than 120 smaller localities in both semi-urban and slum areas were examined. Chi-square test ($\chi^2$) was used to determine the prevalence, while logistic regression was used to compute for adjusted odd ratios. The overall prevalence of leprosy was 33.9 per 10,000 population (range, 9.7–40.7), whereas the new case detection rate was 28.2 per 10,000 (range, 9.7–30.7). Among children <15 years, the leprosy prevalence was 4.4 per 10,000. Adult males aged >15 years had a significantly higher prevalence rate than females of similar age group (92.0 versus 41.6 per 10,000). It was noted that the prevalence rates increased with increasing age ($p <0.0001$). Moreover, workers engaged in manual work were found to have significantly higher prevalence rates than other workers (94.9 versus 21.3, $p <0.0001$). Of the 204 leprosy cases detected, 84.2% were new cases. Of all the cases, 37.3% were of the multibacillary type. Disabilities of Grade II or higher were observed in 12.7% of all cases, of which 9.4% were new cases.—Tropical Disease Bulletin


Leprosy is primarily a disease of skin and peripheral nerves. Because of nerve function impairment, leprosy patients may develop primary nerve related impairments such as, loss of sensation and weakness or paralysis. These primary impairments may lead to secondary impairments such as ulceration and contractures. Many other diseases and disorders present with similar impairments as seen in leprosy e.g., diabetes and peripheral nerve injuries. Nerve function assessment and ulcer prevention and treatment are areas that have been researched in leprosy but these research findings are not yet commonly known and adopted in diseases and disorders that ‘relate’ to leprosy. Rehabilitation is a relatively new field in medicine and not (well) developed in many developing countries. Rehabilitation requires an integrated approach from different disciplines and professionals. As for other medical specialty fields, rehabilitation demands evidence based practice.—Author’s Abstract


The proliferation of nongovernmental organizations (NGOs) can be considered the result of the inability of the current democratic system to perform all the tasks desired by its citizens. Although NGOs often do quite positive work, they tend to diminish governmental power and are capable of
interfering in the internal affairs of other countries. In this context, there are efforts to control their activities, and this control can produce both negative effects (blocking the defense of human rights) and positive ones (correcting the lack of coordination in the work by NGOs). NGOs working with the control of leprosy have a long history of cooperation with “host” states in Latin America. In the worst cases they work in a vacuum left by the state. In a country like Brazil, where the government prioritizes the control of Hansen disease and community participation in the political process—NGOs generally work “in harmony” with national authorities. The most useful contribution to state efforts has been the technical and financial support for training health personnel, supervision, and awareness-raising campaigns. Thus, the NGO becomes “quasi-governmental” in performing its tasks.—Author’s Abstract

Other Mycobacterial Diseases


Nontuberculous mycobacteria are ubiquitous in the environment. Immunocompetent children are commonly infected by these resilient organisms. Cervical lymphadenitis, the most frequent head and neck manifestation of NTM infection, often presents as chronic, unilateral lymphadenopathy with characteristic violaceous overlying skin changes. Diagnosis is ultimately dependent on culture or histopathologic examination of specimen obtained through excisional lymph node biopsy or FNA. The principal treatment of NTM infection remains the surgical excision of diseased tissue. Antibiotics augment surgical therapy and their potential role as a single-modality therapy continues to be investigated.—Authors’ Abstract


Mycobacterium marinum infections cause tenosynovitis of the distal upper extremities and develop as a consequence of skin abrasions acquired in contaminated water. We report on two patients whose MR imaging studies showed tenosynovitis of the distal upper extremity secondary to M. marinum. In one patient sequential MR imaging showed development of bony erosions. Appropriate treatment was delayed in both patients because the diagnosis was not considered. We report on and discuss the clinical course and MR imaging findings in two patients with M. marinum infection.—Authors’ Abstract


Mycobacterium marinum is emerging as an important human pathogen in the United States. We report four cases incidentally diagnosed from culture of biopsy specimens of wrist lesions at a Connecticut inner city hospital between 1996 and 1999. There was no clear association with aquatic exposure and only one patient recalled prior trauma. All were successfully treated with ethambutol and rifampicin. The current literature on the epidemiology, clinical characteristics and management of Mycobacterium marinum infections is reviewed.—Authors’ Abstract


A 58-year-old woman was first seen in November 1999 with a 4-week history of
several tender, deep red or purple, suppurating subcutaneous nodules on the skin of the abdomen, suggestive of a panniculitis. She had no history of systemic immunosuppression. Three months prior to examination, the patient had treated with acupuncture for obesity. Two biopsy specimens of the nodules were taken and sent for culture and histologic examination. Histology showed a pattern of panniculitis with chronic inflammatory cells mixed with areas of polymorphonuclear abscesses and necrosis. Culture of the biopsy specimen grew acid-fast bacilli within 4 days, later identified with biochemical and molecular tests as *Mycobacterium chelonae* (subspecies *chelonae*). Polymerase chain reaction-restriction enzyme pattern analysis (PRA) was used for molecular identification of mycobacteria. *In vitro* sensitivity tests showed sensitivity to clarithromycin, amikacin, tobramycin, doxycycline, and erythromycin, and resistance to ciprofloxacin, ofloxacin, trimethoprim-sulfamethoxazole, imipenem, and cefoxitin. Oral clarithromycin (500 mg b.d.) was started and after 3 months of therapy, the lesions had cleared completely.—Authors’ Abstract


*Mycobacterium avium* subsp. paratuberculosis (*M. paratuberculosis*) enters intestinal epithelial cells of cattle and other ruminants via a mechanism that remains to be fully elucidated. This study showed that a gene encoding the *M. paratuberculosis* 35 kDa major membrane protein (MMP) is expressed at a higher level in low-oxygen and high-osmolarity conditions that are similar to the environment of the intestine. In addition, cattle with Johne’s disease produced antibodies against MMP, suggesting that the protein is present during infection. The gene encoding MMP was cloned and expressed as a fusion protein with the maltose-binding protein (MBP-MMP) in *Escherichia coli*. Rabbit antisera were raised against a *M. paratuberculosis* whole-cell sonicate and MMP-specific antibodies were purified from these sera by affinity chromatography. MMP was localized to the surface of *M. paratuberculosis* by immunoelectron microscopy and by immunoblot analysis of fractionated protein lysates. Both anti-MMP antibodies and MBP-MMP protein inhibited *M. paratuberculosis* invasion of cultured Madin-Darby bovine kidney cells by 30%. In similar invasion experiments with *M. paratuberculosis* incubated in low oxygen tension, these antibodies and protein decreased invasion by 60%. Collectively, these data show that the 35 kDa MMP is a surface exposed protein that plays a role in invasion of epithelial cells. The authors suggest that the MMP is a virulence factor of *M. paratuberculosis* that may be important in the initiation of infection *in vivo*.—Authors’ Abstract


*Mycobacterium avium* subsp. paratuberculosis is a robust and phenotypically versatile pathogen which causes chronic inflammation of the intestine in many species, including primates. *M. avium* subsp. paratuberculosis infection is widespread in domestic livestock and is present in retail pasteurized cows’ milk in the United Kingdom and, potentially, elsewhere. Water supplies are also at risk. The involvement of *M. avium* subsp. paratuberculosis in Crohn’s disease (CD) in humans has been uncertain because of the substantial difficulties in detecting this pathogen. In its Ziehl-Neelsen staining-negative form, *M. avium* subsp. paratuberculosis is highly resistant to chemical and enzymatic lysis. The present study describes the development of optimized sample processing and DNA extraction procedures with fresh human intestinal mucosal biopsy specimens which ensure access to *M. avium* subsp. paratuberculosis DNA and maximize detection of these low-
abundance pathogens. Also described are two nested PCR methodologies targeted at IS900, designated IS900[LV] and IS900[TJ1-4], which are uniquely specific for IS900. Detection of M. avium subsp. paratuberculosis in mucosal biopsy specimens was also evaluated by using mycobacterial growth indicator tube (MGIT) cultures (Becton Dickinson). IS900[LV] PCR detected M. avium subsp. paratuberculosis in 34 of 37 (92%) patients with CD and in 9 of 34 (26%) controls without CD (noninflammatory bowel disease [nIBD] controls) (p = 0.0002; odds ratio = 3.47). M. avium subsp. paratuberculosis was detected by IS900[LV] PCR in MGIT cultures after 14 to 88 weeks of incubation in 14 of 33 (42%) CD patients and 3 of 33 (9%) nIBD controls (p = 0.0019; odds ratio = 4.66). Nine of 15 (60%) MGIT cultures of specimens from CD patients incubated for more than 38 weeks were positive for M. avium subsp. paratuberculosis. In each case the identity of IS900 from M. avium subsp. paratuberculosis was verified by amplicon sequencing. The rate of detection of M. avium subsp. paratuberculosis in individuals with CD is highly significant and implicates this chronic enteric pathogen in disease causation.—Authors’ Abstract


Activities of clarithromycin alone and in combination with rifampicin, gatifloxacin or linezolid were evaluated against Mycobacterium kansasii in a murine infection model. Clarithromycin was the most active single agent. Rifampicin and gatifloxacin had similar activities, but were less active than clarithromycin. Clarithromycin in combination with rifampicin was the most active combination therapy.—Authors’ Abstract


It has been argued whether bronchiectasis is truly caused by MAC infection or just a predisposed condition in which MAC colonizes. Our present study was designed to evaluate the pathological findings of bronchiectases caused by Mycobacterium avium intracellulare complex (MAC) lung infection and to demonstrate MAC in the lesion of bronchiectases. A retrospective study was performed in nine cases with positive cultures for MAC in whom lung resections were performed. A determination of whether or not MAC caused pulmonary disease was made using the 1997 criteria required by the American Thoracic Society. In addition, MAC were cultured from all nine lung specimens. Pathological findings of bronchiectases were evaluated in these nine patients. Destruction of bronchial cartilage and smooth muscles layer, obstruction of airway by granulomas, and ulceration of bronchial mucosa were frequently observed. Our present study demonstrates that destruction of fundamental bronchial structure due to extensive granuloma formation throughout the airways was likely the main cause of bronchiectases in MAC infection.—Authors’ Abstract


Mycobacterium gordonae is historically viewed as an organism with low pathogenic potential, but it has increasingly become implicated in clinical disease in immunocompromised hosts. Illness related to M. gordonae infection ranges from localized infections to rare cases of disseminated disease. This report describes treatment of the first case of occult M. gordonae bacteremia in an adolescent with AIDS.—Authors’ Abstract

Because of the emergence of Buruli ulcer disease, the World Health Organization launched a Global Buruli Ulcer Initiative in 1998. This indolent skin infection is caused by Mycobacterium ulcerans. During a study of risk factors for the disease in Ghana, adequate excisional skin-biopsy specimens were obtained from 124 clinically suspicious lesions. Buruli ulcer disease was diagnosed in 78 lesions since acid-fast bacilli (AFB) were found by histopathologic examination. Lesions with other diagnoses included filariasis (3 cases), zygomycosis (2 cases), ulcerative squamous cell carcinomas (2 cases), keratin cyst (1 case), and lymph node (1 case). Thirty-seven specimens that did not show AFB were considered suspected Buruli ulcer disease cases. Necrosis of subcutaneous tissues and dermal collagen were found more frequently in AFB-positive specimens compared with specimens from suspected case-patients (p <0.001). Defining histologic criteria for a diagnosis of Buruli ulcer disease is of clinical and public health importance since it would allow earlier treatment, leading to less deforming sequelae.—Author’s Abstract


Infections with atypical mycobacteria in Australia during 2000 occurred at a rate of 1.8 cases per 100,000 population. The main sites of disease were the respiratory tract, soft tissue, and the lymphatics. The Mycobacterium avium complex was the most common group of mycobacteria isolated from respiratory, lymphatic sites, and blood. The rapidly growing mycobacteria, predominantly the M. fortuitum-M. abscessus-M. chelonae group were the most common soft tissue infections. Atypical mycobacteria were isolated from significant numbers of sputum ‘smear positive’ patients, requiring further tests to exclude M. tuberculosis. Geographical differences were observed for some Mycobacterium species, notably the isolation of M. haemophilum from Western Australia, and M. ulcerans from Victoria and Queensland. Newer molecular techniques, while improving precision and accuracy of identification, raise additional questions about the ecology of the atypical mycobacteria and their role in disease.—Author’s Abstract


Interferon-gamma receptor-1 (IFNgammaR1) deficiency is a rare inherited immunodeficiency. We performed a nonmyeloablative allogeneic stem cell transplantation on a boy with complete IFNgammaR1 deficiency and refractory disseminated Mycobacterium avium infection. Despite the patient’s profound immune defect, early donor stem cell engraftment was low. Full donor engraftment was accomplished only following multiple donor lymphocyte infusions. Detection of IFNgammaR1 expression on peripheral blood monocytes and neutrophils corresponded with establishment of stable, complete donor hematopoietic chimerism. However, expression of, and signaling through IFNgammaR1 disappeared shortly thereafter. Disseminated Mycobacterium avium infection persisted and the patient died. Coculture of Mycobacterium avium with normal myeloid cells resulted in an IFNgamma signaling defect similar to that observed in vivo. Active disseminated Mycobacterium avium infection may significantly compromise normal immune reconstitution following allogeneic stem cell transplantation. Patients with IFNgammaR1 deficiency should receive transplants.
before developing refractory mycobacterial infections.—Authors’ Abstract


A 25-year-old Thai housewife had a history of tuberculosis of the lymph nodes for six years that had been successfully treated with a course of anti-TB drugs. She developed several red, circumscribed, infiltrative plaques composed of umbilicated papules and pustules on her face and upper part of the body with cervical lymphadenopathy six months later. A pus smear from the lesion grew acid fast bacilli (AFB). Histopathological examination showed a mixed cell granuloma suggestive of infection. A T cell study showed a low CD4 count, and multi skin tests indicated cutaneous anergy. Culture from a biopsy specimen taken from the skin lesion grew M. chelonae; the cultures from blood, urine, and bone marrow. The lesions were not responsive to an anti TB drug given for 2 months based upon the results of the AFB positive pus smear before the culture and sensitivity reports were obtained. Since then the patient was treated with antibiotics according to the results of the sensitivity tests. A combination of amikacin and clarithromycin was started and hyperthermic therapy was later added with a partial response. Based upon the sensitivity test, kanamycin was introduced but had to be stopped because of ototoxicity. Sparfloxacin was used with an effective result but was discontinued for economic reasons. Finally, clarithromycin in combination with clofazimine and cryotherapy were given for a year before the lesions healed completely. It took a three years duration for the total course of treatment for this patient. She is still in remission after two years of follow-up period. This extensive cutaneous M. chelonae infection needed a prolonged combination of antibiotics with the addition of cryotherapy for the non-responsive lesions.—Authors’ Abstract


Spindle cell pseudotumors may occur due to mycobacterial infection in immunocompromised hosts, particularly those with acquired immunodeficiency syndrome (AIDS). Most of the reported mycobacterial spindle cell pseudotumors were found in the lymph nodes. We report a case of spindle cell pseudotumor in a 37-year-old man with AIDS who presented with a firm nodule over his right arm. Histologically, the tumor was composed of proliferative spindle cells admixed with histiocytes and inflammatory cells. Ziehl-Neelsen stain revealed many acid-fast bacilli in the spindle cells and histiocytes. The acid-fast bacilli were shown to be Mycobacterium avium intracellulare by culture and sequencing of the polymerase chain reaction product of mycobacterial 65-kDa heat shock protein gene. Immunohistochemically, the spindle cells were reactive to CD68, suggesting macrophage differentiation of these cells. It is important for pathologists to recognize this unusual manifestation of mycobacterial infection in immunocompromised patients and avoid mistaking the lesion for a mesenchymal neoplasm.—Authors’ Abstract


PURPOSE: To delineate the clinico-pathologic features of patients who have atypical mycobacterial infections of the periorbital region after periocular and facial surgery and to define the sequelae after treatment and their management. METHODS: A case series of patients from 7 practices of ophthalmic plastic and reconstructive surgeons was analyzed retrospectively. RESULTS: Thirteen patients had infection in the following clinical settings: 8 patients had infections after blepharoplasty, 2 patients had infections that involved the anophthalmic socket, 1 patient had orbital
cellulitis after orbital fracture repair with an alloplastic implant, and 2 patients had infections involving the lacrimal system, one after silicone tube insertion and the other after dacryocystorhinostomy with silicone tube intubation. Sequelae of infection included eyelid retraction and ectropion requiring surgical repair (two patients) and enophthalmos (one patient). Twelve of 13 patients required extensive antibiotic therapy. One infection resolved after local excision of eyelid lesions. Another patient had recurrent infection after 4 weeks of antibiotic treatment. CONCLUSIONS: Delayed infection with erythematous nodules, particularly when a foreign body is implanted weeks after periocular surgery, should arouse suspicion of an atypical mycobacterial infection. Delayed infection after blepharoplasty may mimic a chalazion, develop in a sutured incision, or occur without any inflammatory signs. Orbital abscess formation may occur in the setting of transconjunctival blepharoplasty. Cultures for acid-fast bacilli and excisional biopsy of nodules with performance of acid-fast stains may be necessary for diagnosis. The selection of systemic antibiotic therapy, usually clarithromycin, and the length of treatment should be guided by results of culture and sensitivity laboratory studies, biopsy results, and clinical response to treatment. Surgical removal of any implanted foreign bodies should be performed expeditiously. Consultation with an infectious disease specialist may be useful in selected cases. Sequelae of infection may include eyelid scarring and retraction and enophthalmos.—Authors’ Abstract

Molecular & Genetic Studies


In a systematic approach to understand the transcriptional machinery of mycobacteria, we had previously isolated and characterized mycobacterial promoter regions. In this study, we have investigated molecular interactions between mycobacterial RNA polymerase holoenzyme, reconstituted with different sigma subunits and the promoter element of the Mycobacterium tuberculosis gene pknH (Rv1266c), a representative of promoters belonging to the ‘extended -10’ class. In vitro transcription assays using the pknH promoter and reconstituted RNA polymerase holoenzyme demonstrated that transcription from the pknH promoter is specifically initiated by sigmaA, the principal sigma factor of mycobacteria. DNase I protection assay and deletion studies with the pknH promoter revealed that the minimal region required for optimal transcription carries the sequence from position –37 to position +6. Moreover, mutation in the TGN motif of the pknH promoter resulted in the loss of >75% of its activity. Binding of RNA polymerase with wild-type promoter as well as its TG- mutant revealed that the TGN motif is required for the transition from a close complex into an open complex. Further, it was observed that the presence of the TGN motif reduces the thermal energy required for the conversion of a close complex into an open complex, necessary for initiation of transcription.


Tuberculosis in seals is caused by a member of the Mycobacterium tuberculosis complex referred to as the ‘seal bacillus.’ Fluorescent amplified-fragment length polymorphism (FAFLP) analysis was applied to isolates from four Australian and
Current Literature, Molecular and Genetic Studies

Contributions to our understanding of the genetic diversity of mycobacterial species have been made through molecular and genetic studies. For instance, FAFLP (Fluorescent Amplified Fragment Length Polymorphism) profiles have been used to differentiate strains belonging to the *M. tuberculosis* complex. These profiles are derived from EcoRI/MseI restricted fragments of blind coded DNA samples. The FAFLP profiles allow for the discrimination of seal bacilli from other members of the *M. tuberculosis* complex. According to phylogenetic analysis, seal bacilli appear to have diverged significantly from other members of the *M. tuberculosis* complex. This suggests that these bacilli have a unique taxonomic position within the *M. tuberculosis* complex.

The authors describe the suitability of a panel of 19 highly polymorphic markers for rapid identification and comparative genomics of seal bacillus strains. It is likely that these bacilli got separated from the *M. tuberculosis* lineage as a result of different insertion deletion events occurring on a genome wide scale. The authors’ analysis supports the hypothesis that seal bacillus occupies a unique taxonomic position within the *M. tuberculosis* complex.


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The pncA genes in mycobacteria are responsible for the production of pyrazinamidase (PZase). In *Mycobacterium tuberculosis*, PZase hydrolyses pyrazinamide (PZA) to pyrazonic acid, a compound that possesses bactericidal activity against tubercle bacilli. Nucleotide sequences of pncA genes found within mycobacteria were aligned in an effort to ascertain the significance of any variability in sequence. Three sets of primers (one degenerate and five consensus sequences) were designed and employed in a multiplex PCR assay to amplify the pncA region in seven clinically common mycobacteria. The banding patterns generated from each species in conjunction with PZase activity tests demonstrated that the mycobacterial species examined could be clearly identified and differentiated from one another. Although not yet tested with clinical isolates, the combination of these two assays has provided a promising discriminatory tool for the identification of commonly encountered clinical mycobacteria species.—Authors’ Abstract


Unstable variants of green fluorescent protein (GFP) tagged with C-terminal extensions, which are targets for a tail specific protease, have been described in *Escherichia coli* and *Pseudomonas putida* [Appl. Envir. Microbiol. 64 (1998) 2240]. We investigated whether similar modifications to flow cytometer optimized GFP (GFPmut2) could be used to generate unstable variants of GFP for gene expression studies in mycobacteria. We constructed GFP variants in a mycobacterial shuttle vector under the control of the regulatory region of the inducible *Mycobacterium smegmatis* acetamidase gene. GFP expression was induced by the addition of acetamide and the stability of the GFP variants in *M. smegmatis*, following the removal of the inducer to switch off their expression, was determined using spectrofluorometry and flow cytometry. We demonstrate that, compared to the GFPmut2 (half-lives >7 days), the modified GFP variants exhibit much lower half-lives (between 70 and 165 min) in *M. smegmatis*. To investigate their utility in the measurement of mycobacterial gene expression, we cloned the promoter region of a putative amino acid efflux pump gene, lysE (Rv1986), from *Mycobacterium tuberculosis*. It is likely that these bacilli got separated from the *M. tuberculosis* lineage as a result of different insertion deletion events occurring on a genome wide scale. Our analysis reveals that the seal bacillus and *M. bovis* are genetically related and therefore, might have originated from a common ancestor. Our data additionally support the hypothesis that seal bacillus occupies a unique taxonomic position within the *M. tuberculosis* complex.—Authors’ Abstract

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The pncA genes in mycobacteria are responsible for the production of pyrazinamidase (PZase). In *Mycobacterium tuberculosis*, PZase hydrolyses pyrazinamide (PZA) to pyrazonic acid, a compound that possesses bactericidal activity against tubercle bacilli. Nucleotide sequences of pncA genes found within mycobacteria were aligned in an effort to ascertain the significance of any variability in sequence. Three sets of primers (one degenerate and five consensus sequences) were designed and employed in a multiplex PCR assay to amplify the pncA region in seven clinically common mycobacteria. The banding patterns generated from each species in conjunction with PZase activity tests demonstrated that the mycobacterial species examined could be clearly identified and differentiated from one another. Although not yet tested with clinical isolates, the combination of these two assays has provided a promising discriminatory tool for the identification of commonly encountered clinical mycobacteria species.—Authors’ Abstract
bacterium tuberculosis together with the divergently transcribed, putative lysR-type regulator gene (Rv1985c) upstream of one of the unstable GFP variants. We found that the expression kinetics of the lysRE-gfp fusion were identical throughout the M. smegmatis growth curve to those measured using a conventional lysRE-xylE reporter fusion, peaking upon entry into stationary phase. In addition, it was established that the tagged GFP variants were also unstable in Mycobacterium bovis BCG. Thus, we have demonstrated that unstable GFP variants are suitable reporter genes for monitoring transient gene expression in fast- and slow-growing mycobacteria.—Authors’ Abstract


Bacterial genomics revealed the widespread presence of eukaryotic-like protein kinases and phosphatases in prokaryotes, but little is known on their biochemical properties, regulation mechanisms and physiological roles. Here we focus on the catalytic domains of two trans-membrane enzymes, the Ser/Thr protein kinase PknB and the protein phosphatase PstP from Mycobacterium tuberculosis. PstP was found to specifically dephosphorylate model phospho-Ser/Thr substrates in a Mn2+-dependent manner. Autophosphorylated PknB was shown to be a substrate for PstP and its kinase activity was affected by PstP-mediated dephosphorylation. Two threonine residues in the PknB activation loop, found to be mostly disordered in the crystal structure of this kinase, namely Thr171 and Thr173, were identified as the target for PknB autophosphorylation and PstP dephosphorylation. Replacement of these threonine residues by alanine significantly decreased the kinase activity, confirming their direct regulatory role. These results indicate that, as for eukaryotic homologues, phosphorylation of the activation loop provides a regulation mechanism of mycobacterial kinases and strongly suggest that PknB and PstP could work as a functional pair in vivo to control mycobacterial cell growth.—Authors’ Abstract


By the low-stringency single-specific-primer PCR technique, a highly sensitive and rapid method for diagnosis of rifampin resistance in Mycobacterium tuberculosis was established. Seven rifampin-resistant and five rifampin-susceptible specimens were analyzed. Rifampin resistance was determined by MIC measurement. A complex electrophoretic pattern consisting of many bands was obtained for both susceptible and rifampin-resistant isolates. The same pattern was obtained for all of the susceptible specimens, but differences between resistant and susceptible isolates were found. DNA sequencing showed that a particular mutation produces a specific electrophoretic pattern.—Authors’ Abstract


OBJECTIVE: To determine whether 3′UTR polymorphisms of the NRAMP1 gene are associated with tuberculosis in Hans. METHODS: 3′UTR polymorphisms of NRAMP1 gene were typed by PCR-RFLP among 147 patients with active tuberculosis and 145 healthy individuals. The relationship between 3′UTR polymorphisms and susceptibility to tuberculosis was studied, and cases were grouped according to genotypes. RESULTS: In the tu-
Current Literature, Molecular and Genetic Studies

Burckulosis patients, genotype TGTG/TGTG, TGTG/TGTG deleted, and TGTG deleted/TGTG deleted were observed in 95, 50 and 2 cases respectively, while the genotypes of the healthy controls were TGTG/TGTG in 115, TGTG/TGTG deleted in 29 and TGTG deleted/TGTG deleted in 1 case. The frequency of the genotype TGTG/TGTG was found more often among controls than that in patients (chi(2) = 7.79, p <0.01). The frequency of allele TGTG and the frequency of variant allele were 0.85 and 0.15 respectively. CONCLUSIONS: 3'UTR polymorphisms of NRAMP1 gene are associated with susceptibility to tuberculosis in Hans. The variant allele observed in Hans is more common than that in Caucasians. These observations might explain in part why Hans have greater susceptibility to tuberculosis than Caucasians.—Authors’ Abstract


See Current Literature, Immuno-pathology, p. 399


The usefulness of single-enzyme amplified-fragment length polymorphism (AFLP) analysis for the subtyping of *Mycobacterium kansasii* type I isolates was evaluated. This simplified technique classified 253 type I strains into 12 distinct clusters. The discriminating power of this technique was high, and the technique easily distinguished between the epidemiologically unrelated control strains and our clinical isolates. Overall, the technique was relatively rapid and technically simple, yet it gave reproducible and discriminatory results. This technique provides a powerful typing tool which may be helpful in solving many questions concerning the reservoirs, pathogenicities, and modes of transmission of these isolates.—Authors’ Abstract


The RecA-like proteins constitute a group of DNA strand transfer proteins ubiquitous in eubacteria, eukarya, and archaea. However, the functional relationship among RecA proteins is poorly understood. For instance, *Mycobacterium tuberculosis* RecA is synthesized as a large precursor, which undergoes an unusual protein-splicing reaction to generate an active form. Whereas the precursor was inactive, the active form promoted DNA strand transfer less efficiently compared to EcRecA. Furthermore, gene disruption studies have indicated that the frequencies of allele exchange are relatively lower in *Mycobacterium tuberculosis* compared to *Mycobacterium smegmatis*. The mechanistic basis and the factors that contribute to differences in allele exchange remain to be understood. Here, we show that the extent of DNA strand transfer promoted by the *M. smegmatis* RecA in vitro differs significantly from that of *M. tuberculosis* RecA. Importantly, *M. smegmatis* RecA by itself was unable to promote strand transfer, but cognate or noncognate SSBs rendered it efficient even when added prior to RecA. In the presence of SSB, MsRecA or MtRecA catalyzed strand transfer between ssDNA and varying lengths of linear duplex DNA with distinctly different pH profiles. The factors that were able to suppress the formation of DNA networks greatly stimulated strand transfer reactions promoted by MsRecA or MtRecA. Although the rate and pH profiles of dATP hydrolysis catalyzed by MtRecA and MsRecA were similar, only MsRecA was able to couple dATP hydrolysis to DNA strand transfer. Together, these results provide insights into the functional diversity in DNA strand transfer promoted by RecA proteins of pathogenic and nonpathogenic species of mycobacteria.—Authors’ Abstract

The last few years have seen the development of several molecular designs to search for mutations encoding resistance to antituberculous drugs in Mycobacterium tuberculosis. Most of these are highly efficient for RIF-r detection and are well adapted to search for the most relevant INH-R mutations. In this review, these new molecular approaches are explained and are presented according to the molecular strategies on which they are based. In this sense, techniques based on DNA-sequencing, electrophoresis and hybridization are reviewed and the newer designs based on real-time PCR and microarrays are also included. Molecular methods are sure to transform standard approaches to the issue of resistance in the mycobacteriology laboratory. This will allow laboratories to speed up the performance of resistance assays and provide access to essential information for highly refined detection, follow-up and management of antibiotic resistance in M. tuberculosis.—Author’s Abstract


The dimannoside (PIM2) and hexamannoside (PIM6) phosphatidyl-myo-inositol mannosides are the two most abundant classes of PIM found in Mycobacterium bovis bacillus Calmette Guerin, Mycobacterium tuberculosis H37Rv, and Mycobacterium smegmatis 607. Recently, these long known molecules received a renewed interest due to the fact that PIM2 constitute the anchor motif of an important constituent of the mycobacterial cell wall, the lipoarabinomannans (LAM), and that both LAM (phosphoinositol-capped LAM) and PIM are agonists of Toll-like receptor 2 (TLR2), a pattern recognition receptor involved in innate immunity. Due to the biological importance of these molecules, the chemical structure of PIM was revisited. The structure of PIM2 was recently published (Gilleron, M., Ronet, C., Mempel, M., Monsarrat, B., Gachelin, G., and Puzo, G. (2001) J. Biol. Chem. 276, 34896–34904). Here we report the purification and molecular characterization of PIM6 in their native form. For the first time, four acyl forms of this molecule have been purified, using hydrophobic interaction chromatography. Mono- to tetraacylated molecules were identified in M. bovis bacillus Calmette Guerin, M. tuberculosis H37Rv, and M. smegmatis 607 using a sophisticated combination of analytical tools, including matrix-assisted laser desorption/ionization-time of flight mass spectrometry and two-dimensional homo- and heteronuclear NMR spectroscopy. These experiments revealed that the major acyl forms are similar to the ones described for PIM2. Finally, we show that PIM6, like PIM2, activate primary macrophages to secrete TNF-alpha through TLR2, irrespective of their acylation pattern, and that they signal through the adaptor MyD88.—Authors’ Abstract


An evaluation of the utility of IS6110-based restriction fragment length polymorphism (RFLP) typing compared to a combination of variable number tandem repeat (VNTR) typing and mycobacterial interspersed repetitive unit (MIRU) typing was undertaken. A total of 53 patient isolates of Mycobacterium tuberculosis from four presumed episodes of cross-infection were examined. Genomic DNA was extracted from the isolates by a cetyl trimethylammonium bromide method. The number of copies of tandem repeats of the five loci ETR(A) to
ETR(E) and 12 MIRU loci was determined by PCR amplification and agarose gel electrophoresis of the amplicons. VNTR typing identified the major clusters of strains in the three investigations in which they occurred (each representing a different evolutionary clade: 32333, 42235, and 32433). The majority of unrelated isolates (by epidemiology and RFLP typing) were also identified by VNTR typing. The concordance between the RFLP and MIRU typing was complete, with the exception of two isolates with RFLP patterns that differed by one band each from the rest of the major epidemiologically linked groups of isolates in investigation A. All of these isolates had identical MIRU and VNTR types. A further pair of isolates differed in the number of tandem repeat copies at two MIRU alleles but had identical RFLP patterns. The speed of the combined VNTR and MIRU typing approach enabled results for some of the investigations to be supplied in “real time,” influencing choices in contact tracing. The ease of comparison of results of MIRU and VNTR typing, which are recorded as single multidigit numbers, was also found to greatly facilitate investigation management and the communication of results to health care professionals.—Authors’ Abstract


To examine the virulence factors of *Mycobacterium tuberculosis* H37Rv, the proteome was used to characterize the differences in protein expression between virulent *M. tuberculosis* H37Rv and attenuated *M. tuberculosis* H37Ra. Two-dimensional gel electrophoresis was performed to separate culture supernatant proteins extracted from *M. tuberculosis* H37Rv and *M. tuberculosis* H37Ra. The protein spots of interest were identified by mass spectrometry, and then the genes encoding the identified proteins were cloned and sequenced. Comparison of silver-stained gels showed that three well-resolved protein spots were present in *M. tuberculosis* H37Rv but absent from *M. tuberculosis* H37Ra. Protein spot no. 1 was identified as Rv2346c. Protein spot no. 2 was identified as Rv2347c, Rv1197, Rv1038c, and Rv3620c, which shared significant homology and had the same peptide fingerprinting using tryptic digestion. No *M. tuberculosis* protein matched protein spot no. 3. Rv2346c, Rv2347c, Rv1038c, and Rv3620c of *M. tuberculosis* H37Rv were located on the *M. tuberculosis* H37Ra chromosome, and multiple mutations were observed in the corresponding areas of *M. tuberculosis* H37Ra. Codon 59 (CAG, Gln) of Rv2347c and Rv3620c was replaced by termination codon (TAG) in *M. tuberculosis* H37Ra, which probably terminated the polypeptide elongation. These results demonstrate the importance of studying the gene products of *M. tuberculosis* and show that subtle differences in isogenic mutant strains might play an important role in identifying the attenuating mutations.—Authors’ Abstract


Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method in which reagents react under isothermal conditions with high specificity, efficiency, and rapidity. We used LAMP for detection of *Mycobacterium tuberculosis* complex, *Mycobacterium avium*, and *Mycobacterium intracellulare* directly from sputum specimens as well as for detection of culture isolates grown in a liquid medium (MGIT; Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) or on a solid medium (Ogawa’s medium). Species-specific primers were designed by targeting the gyrB gene, and their specificities were validated on 24 mycobacterial species and 7 nonmycobacterial species. The whole procedure is quite simple, starting with the mixing of all reagents in a single tube, followed by an isothermal reaction during which the reaction mixture is held at 63 degrees C. The resulting amplicons are visualized by adding SYBR Green I
to the reaction tube. The only equipment needed for the amplification reaction is a regular laboratory water bath or heat block that furnishes a constant temperature of 63 degrees C. The assay had a detection limit of 5 to 50 copies of purified DNA with a 60-min incubation time. The reaction time could be shortened to 35 min for the species identification of *M. tuberculosis* complex, *M. avium*, and *M. intracellularare* from a solid-medium culture. Residual DNA lysates prepared for the Amplicor assay (Roche Diagnostics GmbH) from 66 sputum specimens were tested in the LAMP assay. Although the sample size used for the latter assay was small, 2.75 micro l of the DNA lysates, it showed a performance comparable with that of the Amplicor assay, which required 50 micro l of the lysates. This LAMP-based assay is simple, rapid, and sensitive; a result is available in 35 min for a solid-medium culture and in 60 min for a liquid-medium culture or for a sputum specimen that contains a corresponding amount of DNA available for testing.—Authors’ Abstract


Commonly, 16S ribosome RNA (16S rRNA) sequence analysis has been used for identifying enteric bacteria. However, it may not always be applicable for distinguishing closely related bacteria. Therefore, we selected gyrB genes that encode the subunit B protein of DNA gyrase (a topoisomerase type II protein) as target genes. The molecular evolution rate of gyrB genes is higher than that of 16S rRNA, and gyrB genes are distributed universally among bacterial species. Microarray technology includes the methods of arraying cDNA or oligonucleotides on substrates such as glass slides while acquiring a lot of information simultaneously. Thus, it is possible to identify the enteric bacteria easily using microarray technology. We devised a simple method of rapidly identifying bacterial species through the combined use of gyrB genes and microarrays. Closely related bacteria were not identified at the species level using 16S rRNA sequence analysis, whereas they were identified at the species level based on the reaction patterns of oligonucleotides on our microarrays using gyrB genes.—Authors’ Abstract


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We sequenced a 516 base pair segment in the 23S rRNA gene of 54 *Mycobacterium tuberculosis* isolates, 52 of which were clinical isolates from Ethiopia. Sequence polymorphism was observed with 19 of the 54 strains; the polymorphic sites occurred in less than 2% (9/516) of the total sequence positions. The sequence variations represented base pair substitutions (14/23), insertions (9/23) or both (1/23). Insertions occurred at one site only, whereas substitutions were observed in various regions of the gene. There was no relation between mutational sites and drug susceptibility. However, using information from the GenBank database, comparison between the 23S rDNA sequences of *M. tuberculosis* and the corresponding sequences of other mycobacteria and of related non-mycobacterial species revealed considerable variation, suggesting that this region may provide a target for rapid detection and identification of mycobacteria both at the genus or species level.—Authors’ Abstract
The Editor, on behalf on the INTERNATIONAL JOURNAL OF LEPROSY and the membership of the International Leprosy Association, expresses his deepest appreciation to the following reviewers who have provided invaluable expertise, criticism, and advice in preparing Volume 71.

Linda Adams
Warwick Britton
Anne Burdick
Paul Converse
Richard Croft
Mannam Ebenezer
Richard Frankel
Thomas Gillis
Shigeo Hashimoto
Robert Hastings
Carlotta Hill
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Diana Williams
ACKNOWLEDGMENT

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

SPECIAL GRANTORS

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

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

*Amici dei Lebbrosi, Fondazione Italiana Raoul Follereau, Via Borselli 4, 40135 Bologna, Italy.

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

*Damien Foundation (DF/APD), 16 Rue Stevin, B-1040 Bruxelles, Belgium.

*Deutsches Aussatzigen-Hilfswerk e. V., Postfach 9062, D-97090 Würzburg 11, Germany.

*Le Secours aux Lépreux (Canada), 1275 Rue Hodge, Bureau 12, Montreal H4N 3H4, Canada.

*Netherlands Leprosy Relief, Wibautstraat 137K, 1097 DN Amsterdam, The Netherlands.

*Pacific Leprosy Foundation, 115 Sherborne Street, Bag 4730, Christchurch, New Zealand.


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