Images from the History of Leprosy

Previous page: United States Public Health Service Hospital, Carville, Louisiana, 1936.

Reproduced here is a photograph of the original wooden structures, taken by Charles Marshall, a patient and photographer, which shows the residential buildings in the foreground and the hospital building in the upper right, all connected by covered walkways. This design was emulated at many institutions around the world in the attempt to provide better accommodations and medical facilities to patients. Even today, some institutions in other countries refer to similar residential dormitories as “Carvilles.”

The image here is electronically reproduced from an original black and white print measuring 3.5 × 5 inches. The photo is provided courtesy of the National Hansen’s Disease Museum, Carville, LA, www.bphc.hrsa.gov/nhdp/NHD_MUSEUM_HISTORY.htm
An Approach to Understanding the Transmission of *Mycobacterium leprae* Using Molecular and Immunological Methods: Results from the MILEP2 Study


**ABSTRACT**

**Background.** The current strategy for leprosy control using case detection and treatment has greatly reduced the prevalence of leprosy, but has had no demonstrable effect on interrupting transmission.

**Methods.** Three leprosy endemic communities in India were recruited, examined, and followed up sequentially over 2 yrs using nasal swabs and saliva collections. The nasal swabs were tested by polymerase chain reaction for the presence of *M. leprae* and the saliva was assayed for anti-*M. leprae* IgA.

**Findings.** Only 1.6% of 2552 nasal swabs were PCR positive, and 68% of saliva samples were positive for ML-IgA. BCG and household contact status was associated with the mucosal immune response, but not with PCR positivity. PCR positivity did not persist and most PCR positive results were in the wet season.

**Interpretation.** The findings contribute to our understanding of the epidemiology of *M. leprae* and the possible periods of greatest likelihood of exposure and transmission.
Important developments have taken place over the past decade in tackling the global leprosy burden. There has been a very significant reduction of over 90% in the total number of leprosy patients registered for treatment through the implementation of multidrug therapy regimes (19) that have dramatically reduced the duration of treatment. However, despite this reduction in registered prevalence of leprosy, there has been no parallel reduction in the global case detection (18). This is a serious barrier to aspirations to eradicate leprosy in the future.

Mycobacterium leprae was the first bacterium shown to be associated with disease in humans through the work of Hansen (15) in the 1870s, yet the sources of infection, mode of transmission and early pathogenesis of infection remain an enigma (9). The inability to cultivate M. leprae in vitro and the limitations of in vivo cultivation mean that our current understanding of the transmission is largely based on circumstantial evidence. Observations from studies of household contacts show that households with leprosy patients have higher attack rates of leprosy than those without such exposure, and the attack rates are higher where the index case has a higher bacterial load (7). Despite this, most new patients do not report a history of contact, which raises questions of the nature of the exposure, the pattern of responses, and the possibility of other reservoirs of infection. Careful epidemiological study can demonstrate contacts for most cases in certain settings though most patients may be unaware of such contact (16). The very slow rate of multiplication of M. leprae, the long incubation period (years rather than days) and the variable host immune response make it extremely difficult to link exposure to disease
outcome. Earlier work clearly demonstrated that the nose is a major route of exit for *M. leprae* (13), and the nose may also be a major route of entry (14).

The immunological response to exposure to *M. leprae* is complex. Measurements of cell-mediated immunity are the most relevant in leprosy, as *M. leprae* is an intracellular pathogen, however findings are difficult to interpret (2). However it has been postulated that if the earliest lesions are in the nasal mucosa then mucosal IgA responses may be the most important markers of early exposure and infection (1). Recent methodological developments now make it possible to study these mucosal responses in populations (10).

Improved understanding of the transmission of leprosy is an important key to developing approaches that may interrupt transmission, leading to eradication, rather than the current approach of disease control based on early case detection and treatment (4). The development of genetic and molecular methods creates the potential to address some of these critical questions about transmission. Polymerase chain reaction (PCR) methods have been used in the study of *M. leprae* for a number of years but in the early work there were serious concerns about the validity of the methods and detection systems. Recent research, which analyzed nasal swabs taken in the community in leprosy endemic and non-endemic countries along with blinded spiked laboratory samples, has answered many of the earlier concerns about validity of findings (10). Currently, a series of studies in a number of countries have produced interesting findings on the distribution of *M. leprae* in the community that challenge conventional theories. In these studies healthy individuals in leprosy endemic countries have been shown to have nasal swabs that have tested positive for *M. leprae*. The evidence suggests that positivity may persist in communities but not in individuals (8, 11, 12, 17).

In this study on the Mucosal Immunology of Leprosy (MILEP2), we have combined both PCR and immunological techniques to study three communities in India repeatedly over a period of 2 yrs. The aim of the study was to establish the relationship between *M. leprae* infection and the development of immune responses in a community in which multidrug therapy (MDT) had been used for more than 10 yrs and to elucidate the pathogenesis of primary nasal infection in leprosy.

**METHODS**

Three communities in India with similar characteristics and leprosy endemicity were selected for the study to test the consistency in findings and offer the potential to conduct community based intervention studies. The three communities were villages in southern Maharashtra of between 1000 and 1500 population and within a day’s travel from the Stanley Browne Research Laboratory in the Richardson Leprosy Hospital at Miraj, Maharashtra. Prior to commencement of the main study, pilot studies for development of methods and logistical studies were undertaken in a fourth village. The presence of *M. leprae* DNA in the nose of participants was measured by PCR, while mucosal immunity was assessed by enzyme-linked immunosorbent assay (ELISA) for anti-*M. leprae* IgA antibodies.

**Community surveys.** The villages were selected from all villages within the Sangli District where a MDT-based leprosy control program had been in operation for more than 10 yrs. Data on the size, socio-demographic characteristics and leprosy epidemiology of all villages was collated and three villages of roughly equal size (1000 to 1500 population), similar average incidence rate of leprosy over the previous 10 yrs of around 1 per 1000 per year, and physically separate but within a 50 mile radius from the laboratory, were selected. Each village was then visited in turn by the investigators and meetings with the village elders and the village community convened. The basis of the study was explained, the proposed methods described, and agreement for the participation was secured. Thereafter, a census of each village was conducted which included examination of all those present. A number of visits were conducted to achieve a high coverage of each village. Details of the residents were collected by household and these included: age, sex, marital status, occupation, BCG immunization, history of leprosy, previous contact with leprosy, tuberculosis and other diseases. All household members in households with a case of leprosy in the previous 10 yrs were classi-
fied as household contacts. In addition, each resident was clinically examined for signs of leprosy. Nasal swabs (2 specimens per person from right and left nostril) were obtained from all individuals over three years of age for the PCR analysis and a sample of saliva collected to assay mucosal IgA. The survey was repeated on 2 to 3 further occasions over a two-year period, when those present were re-examined for signs of leprosy and further nasal swabs and saliva samples were taken.

**Laboratory methods.** A pilot study was conducted in a separate village to test out the procedures for specimen collection and transportation. Nasal swabs and saliva samples were transported on the same day they were taken, from each of the villages to the laboratory in Miraj. The samples were numbered in the field to link to the survey data and given a unique identifier on receipt of the specimens in the laboratory.

**PCR assays of nasal swabs.** DNA extracted from nasal swabs was analyzed by PCR. A simple method for the rapid screening of PCR samples using the Peptide-Nucleic-Acid (PNA) hybridization technique with an ELISA endpoint was employed and is described in detail in a previous publication (11). This method using Ready-to-Go™ PCR Beads (Pharmacia) was designed specifically to be simple and robust enough to be used in leprosy-endemic areas where there may be difficulties in maintaining a reliable cold chain for the transport and storage of various components.

**Salivary IgA anti-M. leprae (ML-IgA) immunoassay.** A previously developed ELISA method with whole *M. leprae* as antigen was used (14), and stringent statistical criteria were applied to the results to ensure the reproducibility and uniformity of the ELISA and the positivity of the samples for *M. leprae*. The salivary IgA assay is semi-quantitative so the results are presented as negative (−) or positive (+), with those found in the top 10% of the distribution considered highly positive (++)

**Data analysis.** Results from the baseline survey and laboratory assays were analyzed to describe the frequency of PCR and IgA positivity in the three communities and their relationship to demographic variables. The cross sectional data from the baseline survey in all three villages was analyzed to assess the relationship between PCR and IgA results with household contact status, BCG immunization, and seasonal patterns. The data from the two follow-up surveys were analyzed to assess changes in both PCR and IgA status and their persistence over time in individuals and communities. The statistical methods used were differences in proportions and the chi square test.

**Study funding and approval.** The study was funded by the European Commission as part of the Health Research with Developing Countries Program (INCO-DC). The Indian Council for Medical Research granted approval for the study.

**RESULTS**

Surveys were carried out four times in village 1 (between November 1997 and July 1999), three times in village 2 (between March 1998 and May 1999), and three times in village 3 (between July 1998 and September 1999). The findings of the baseline surveys for each of the three villages are presented in Table 1. The total enumerated population of the three villages was 3725 and examinations were conducted in 3034 (81.4%), with slightly higher coverage in females 1513 (84.3%) than in males 1521 (78.8%). Three new cases of leprosy were diagnosed during the surveys (all male and aged 7, 25, and 60 yrs) and overall 6.7% of those surveyed were household contacts of current or previous leprosy patients (46 cases). Overall BCG coverage was 50.1%, and demonstrated an age effect due to the implementation of BCG programs in the under 35 yrs age groups, but 95% coverage in the 10 to 15 yrs age group.

Strict quality control criteria were applied and valid PCR analysis was obtained on 2552 of 3034 (84.1%) samples tested. In the baseline survey, only 42 of these 2552 (1.6%) nasal swabs were found to be PCR positive. There was no clear pattern by age or sex but there was variation by village ranging from 0.1% to 3.3%. In the saliva samples, 2890 out of 3034 (95.2%) met the quality control criteria for the salivary IgA assay. Of those assays meeting the criteria, 67.7% were positive for salivary ML-IgA, this varied from 53% to 81% between villages. This variation was also seen in the top 10% of ML-IgA high titres but no dif-
Table 1. Baseline survey results by village.

<table>
<thead>
<tr>
<th>Village</th>
<th>Enumerated</th>
<th>Examined</th>
<th>% BCG</th>
<th>% Household Contact</th>
<th>Nasal PCR Valid</th>
<th>+ (%)</th>
<th>Salivary IgA Valid</th>
<th>+ (%)</th>
<th>++ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Village 1</td>
<td>Male</td>
<td>792</td>
<td>641</td>
<td>49.6</td>
<td>450</td>
<td>7</td>
<td>1 (1.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>748</td>
<td>657</td>
<td>37.9</td>
<td>457</td>
<td>8</td>
<td>1 (1.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village 2</td>
<td>Male</td>
<td>615</td>
<td>462</td>
<td>58.4</td>
<td>439</td>
<td>1</td>
<td>0 (0.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>561</td>
<td>451</td>
<td>56.5</td>
<td>422</td>
<td>0</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village 3</td>
<td>Male</td>
<td>523</td>
<td>418</td>
<td>61.7</td>
<td>402</td>
<td>15</td>
<td>3 (7.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>486</td>
<td>405</td>
<td>42.2</td>
<td>382</td>
<td>11</td>
<td>2 (2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All villages</td>
<td>Male (Total)</td>
<td>1930</td>
<td>1521</td>
<td>55.6</td>
<td>1291</td>
<td>23</td>
<td>1 (1.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female (Total)</td>
<td>1795</td>
<td>1513</td>
<td>44.6</td>
<td>1261</td>
<td>19</td>
<td>1 (1.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3725</td>
<td>3034</td>
<td>50.1</td>
<td>2552</td>
<td>42</td>
<td>1 (1.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Nasal PCR and salivary ML-IgA in household contacts.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Household contacts</th>
<th>Salivary IgA</th>
<th>Valid</th>
<th>+ ve (%)</th>
<th>++ ve (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household contacts</td>
<td>97</td>
<td>78</td>
<td>1 (1.28)</td>
<td>96</td>
<td>64 (66.7)</td>
<td>18 (18.8)</td>
</tr>
<tr>
<td>Non contacts</td>
<td>1424</td>
<td>1190</td>
<td>22 (1.58)</td>
<td>1313</td>
<td>839 (63.9)</td>
<td>108 (8.2)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household contacts</td>
<td>105</td>
<td>79</td>
<td>0 (0)</td>
<td>105</td>
<td>74 (70.5)</td>
<td>19 (15.2)</td>
</tr>
<tr>
<td>Non contacts</td>
<td>1408</td>
<td>1172</td>
<td>19 (1.62)</td>
<td>1333</td>
<td>949 (71.2)</td>
<td>140 (10.5)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household contacts</td>
<td>202</td>
<td>157</td>
<td>1 (0.6)</td>
<td>201</td>
<td>138 (68.7)</td>
<td>34 (16.9)</td>
</tr>
<tr>
<td>Non contacts</td>
<td>2832</td>
<td>2362</td>
<td>41 (1.7)</td>
<td>2646</td>
<td>1788 (67.6)</td>
<td>248 (9.4)</td>
</tr>
</tbody>
</table>

Differences in proportions

**p <0.01
*p <0.05

There was a highly statistically significant association (p <0.01) between higher ML-IgA and household contact status with higher levels being observed in household contacts, but no evidence of an association between PCR positivity and household contact status (Table 2). A greater proportion (12.5%) of those with no record of BCG immunization had high ML-IgA titres compared to those with BCG (Table 3); this was highly statistically significant (p <0.01). Of the 42 individuals who tested positive for PCR, only 6 (14.3%) were also IgA strongly positive, which was not significantly different from those who were negative for nasal PCR, and 86% of the surveyed population from the 3 villages were negative for both nasal PCR and salivary ML-IgA (Table 4).

There was, however, a seasonal pattern to PCR positivity with a peak between August and November coinciding with the monsoon season (Table 5), with ML-IgA strongly positive results increasing to 37% in November.

Follow up surveys. An 80% response rate was achieved in the baseline surveys conducted in all three villages. The first
follow-up in village 1 was low (25%) due to the selection of subjects who were identified as PCR-positive. This led to some difficulties for these individuals and in subsequent follow-up surveys all available villagers were re-tested regardless of previous PCR status. The participation rate for the first and second follow-up surveys was 43–56% and higher among females than males. The percentage of PCR positive individuals in these two follow-up examinations varied by village, but was consistent with the seasonal pattern observed in the baseline survey (Table 6). High levels of ML-IgA found in saliva tended to follow the periods of PCR positivity. There was also evidence of a lack of persistence of PCR positivity and IgA over time. Of the 42 individuals who were PCR positive in the baseline survey, 24 were successfully tested in the first follow-up survey and 23 in the second follow-up survey. Of these none were positive in the first follow-up survey and only one in the second follow-up survey (Table 7).

**DISCUSSION**

This study is the largest and most comprehensive investigation of the role of the upper respiratory tract in the transmission of *M. leprae* using a combined molecular and immunological approach. This paper represents the culmination of 10 yrs of development work establishing a laboratory in a leprosy endemic country, and the development of robust methods under field conditions to study leprosy transmission. The current global leprosy control strategy, based on early detection of leprosy and treatment using MDT, has had a dramatic effect of the numbers of leprosy patients registered for treatment but has not had a demonstrable effect on interrupting transmission. The approach adopted in this study combines the use of PCR methods to identify small numbers of *M. leprae* with salivary tests for mucosal immune responses to *M. leprae*. The findings of this large scale population based study tracking three communities over a two year period are therefore relevant and timely.

The initial analyses of the PCR and IgA results looked at age and sex distributions, geographical patterns, temporal trends, relationships to BCG immunizations and tuberculosis infections, associations with contact status and the persistence over time of the findings in individuals. The baseline and follow-up surveys revealed that there was considerable variation between the villages in levels of nasal PCR positivity, but there was no clear age or sex pattern, or association with recent leprosy contact or BCG status. Conversely, high ML-IgA responses demonstrated possible associations with

### Table 3. PCR and salivary IgA by BCG status.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>PCR</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Valid</td>
<td>+ ve (%)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCG</td>
<td>846</td>
<td>742</td>
<td>14</td>
</tr>
<tr>
<td>No BCG</td>
<td>676</td>
<td>549</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCG</td>
<td>675</td>
<td>582</td>
<td>8</td>
</tr>
<tr>
<td>No BCG</td>
<td>838</td>
<td>679</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>1521</td>
<td>1324</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>1514</td>
<td>1228</td>
<td>20</td>
</tr>
</tbody>
</table>

Differences in proportions

**p <0.01
*p <0.05

### Table 4. Association between nasal PCR status and salivary ML-IgA.

<table>
<thead>
<tr>
<th>High salivary IgA</th>
<th>Nasal PCR result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ ve</td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
</tr>
<tr>
<td>No</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
</tr>
</tbody>
</table>
TABLE 5. Monthly pattern of nasal PCR and salivary ML-IgA.

<table>
<thead>
<tr>
<th>Month</th>
<th>Nasal swab PCR</th>
<th>Salivary ML-IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valid PCR</td>
<td>+ve</td>
</tr>
<tr>
<td>January</td>
<td>156</td>
<td>0</td>
</tr>
<tr>
<td>February</td>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>April</td>
<td>659</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>146</td>
<td>1</td>
</tr>
<tr>
<td>June</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>July</td>
<td>93</td>
<td>1</td>
</tr>
<tr>
<td>August</td>
<td>604</td>
<td>23</td>
</tr>
<tr>
<td>September</td>
<td>87</td>
<td>2</td>
</tr>
<tr>
<td>October</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>November</td>
<td>370</td>
<td>14</td>
</tr>
<tr>
<td>December</td>
<td>339</td>
<td>1</td>
</tr>
</tbody>
</table>

contact status and lack of BCG immunization.

The major, new finding from this study is the strong seasonal pattern of nasal PCR and high titre ML-IgA positivity. This suggests that there may be high levels of exposure to *M. leprae* within these populations during the wet season. The peak rainfall period in the area of the study being July to October, this would fit with the high ML-IgA levels in November at the end of the wet period. The rates of PCR positive nasal swabs are low at the start of the wet period and continue high in the period immediately after the monsoon rains. It has long been considered that transmission of leprosy infection was easier in a wet environment; *M. leprae* does not survive prolonged drying (5), and the disease is common in tropical areas of high humidity (6). One might then postulate that endemic areas of relatively constant high humidity might not show the same degree of seasonal transmission as appears to be present in Maharashtra. The long incubation period between infection and disease makes it difficult to assess seasonal patterns of transmission from any season pattern in the incidence or indeed the detection of disease. While the possibility of an environmental reservoir cannot be excluded, this may not be necessary if there is sufficient and repeated transient infection of non-immune individuals as we have previously suggested from earlier studies (4). An alternative explanation of the seasonal pattern observed in this study is that of closer contact and increased exposure within the household as a result of longer periods of time spent indoors and poorer ventilation.

The transient nature of nasal PCR positivity is also striking and has been observed in previous studies (8), and it appears to be associated with the development high ML-IgA titres. Studies of the carriage of other bacteria in the upper respiratory tract have

TABLE 6. Percentage nasal PCR and salivary ML-IgA positive at each survey.

<table>
<thead>
<tr>
<th>Village</th>
<th>Nasal PCR</th>
<th>High salivary ML-IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>First follow-up</td>
</tr>
<tr>
<td>Village 1</td>
<td>Male</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.8</td>
</tr>
<tr>
<td>Village 2</td>
<td>Male</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.0</td>
</tr>
<tr>
<td>Village 3</td>
<td>Male</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2.9</td>
</tr>
</tbody>
</table>
shown similar transient occurrence in individuals with persistence in the community (3). Unlike the divergence of cell-mediated and humoral responses seen in the systemic immune response to *M. leprae* infection, mucosal IgA responses appear to be induced with systemic cell-mediated immunity, suggesting that the development of mucosal immunity to *M. leprae* may be protective (4). The data presented here support this but there was no measurement of cell-mediated immune responses in this large epidemiological study.

The authors of this paper are aware that there were a number of limitations of this study since not all of the follow-up surveys were of the same individuals and some of the intervals between surveys were not linked to seasons. It also may be argued that the PCR positivity could be due to the presence of other non-leprosy mycobacteria sharing the same sequences as the primers used in this study. The two primers are considered to be specific to *M. leprae*, based on known sequenced mycobacteria. We used two different sets of specific primers, based on the sequences of the pra gene and the repetitive element RLEP. The processing of large numbers of PCR and IgA assays has demanded the development of rigorous internal and external quality control procedures, and batches failing quality control (up to 50%) were required to be re-run. PCR results need to be interpreted with caution and laboratories need to demonstrate rigorous quality control procedures. External quality control based on nasal swabs from a non-endemic country (10) demonstrates that it is possible to achieve good sensitivity and specificity. The development of these methods and their application to field studies provide tools for studying the transmission of leprosy and direct methods for testing innovative interventions.

Validation of these findings requires careful follow-up a cohort every month for a year to confirm if there is a relationship between the transient nature of PCR positivity and seasonal patterns. Studies of contacts of new untreated patients and studies to investigate environmental sources in the wet season would be of value. It would be possible to examine first exposure by serial examination of new-born infants and previously unexposed visitors to leprosy-endemic areas. Confirmation of the seasonal nature of *M. leprae* infection would have major implications for our understanding of leprosy transmission and for interrupting transmission as it may be possible to protect populations for a relatively short period timed to coincide with the period of greatest likelihood of exposure.

**Contributions.** All the study partners contributed to the development of the study proposal co-ordinated by Smith, the study builds on an earlier developmental program led by Cree and also funded by the European Commission. Cree, Macdonald, and Jadhav developed the PCR methods with guidance and technical advice from Klatser, Van Beers, and Oskam. The PCR quality control was organized and assessed by Oskam and Klatser. The ML-IgA methods were developed by Cree, Jadhav, and Fernando, with guidance and technical advice for Bjune. The fieldwork was designed and developed by Rao, Edward, Macdonald,

<table>
<thead>
<tr>
<th>Baseline PCR Positive</th>
<th>First follow-up PCR results</th>
<th>Second follow-up PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>Positive 0</td>
<td>Positive 1</td>
</tr>
<tr>
<td></td>
<td>Negative 24</td>
<td>Negative 22</td>
</tr>
<tr>
<td></td>
<td>No data 18</td>
<td>No data 19</td>
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</table>

<table>
<thead>
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<th>Salivary ML-IgA results</th>
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<tbody>
<tr>
<td>Baseline ML-IgA</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Negative 13</td>
</tr>
<tr>
<td>ML-IgA + 24</td>
</tr>
<tr>
<td>ML-IgA ++ 5</td>
</tr>
<tr>
<td>No data 0</td>
</tr>
</tbody>
</table>

**Table 7.** Follow-up of the 42 individuals nasal PCR positive at baseline.
and Jadhav with technical advice from W. Smith, C. Smith, and van Beers. Jadhav, W. Smith, and C. Smith conducted the collation of data and analysis. All authors contributed to the drafting process in the preparation of this paper and approved the final version. There are no conflicts of interest.

Acknowledgment. The MILEP2 study group acknowledges the co-operation of the participating village communities in India without whom this study would not have been possible, and also the survey team and laboratory staff and the European partners who have spent time at the study center. The work is funded by the European Commission as part of the International Co-operation with Developing Countries Program (INCO-DC) contract number IC18*CT960047. We are grateful to The Leprosy Mission for their support for this project and for the assistance of their staff in Delhi and at Miraj. We thanks Dr. S. D. Sontakke for her support to the laboratory staff at Miraj. We acknowledge the assistance from Professor Anand Job from CMC Vellore who conducted nasal examinations on those found to be PCR positive. We are grateful to Professor Patrick Brennan, Colorado State University for the free supply of whole M. leprae for the ML-IgA assay.

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Validity of the WHO Operational Classification and Value of Other Clinical Signs in the Classification of Leprosy

Gift Norman, Geetha Joseph, and Joseph Richard

ABSTRACT

The objective of this study is to examine the validity of the WHO operational classification using skin smear results as the gold standard and explore the value of additional clinical signs independently and in combination with the WHO classification.

Between 1985 and 2000, 5439 new untreated leprosy patients were registered at the Schieffelin Leprosy Research and Training Center, Karigiri. They were classified according to the Ridley Jopling classification as well as WHO operational classification based on the number of skin lesions.

The sensitivity and specificity of the WHO operational classification tested, using skin smear results as the gold standard, was found to be 88.6% and 86.7% respectively. The Receiver Operator Characteristic (ROC) curve confirms that the best option for sensitivity and specificity is a cut off of 6 and more lesions for MB.

The validity of the number of enlarged nerves and size of the largest skin lesion as independent criteria to classify patients was found to be poor. Addition of three enlarged trunk nerves to the WHO classification improved its sensitivity to 91.4%, while the specificity remained almost unchanged at 85.3%. Addition of the size of the largest skin lesion to the WHO classification reduced its validity considerably.

The study concludes that the WHO recommendation of using six and more lesions for classifying a patient as MB is the best option available at the moment, and calls for further research to identify other clinical criteria that have a better validity and could be easily applied in the field.

RÉSUMÉ

L’objectif de cette étude est d’examiner la validité de la classification opérationnelle de l’OMS, qui utilise l’examen bactérioscopique de frottis de suc dermique comme méthode de référence, et d’explorer l’intérêt d’intégrer des signes cliniques additionnels, indépendamment ou bien en combinaison avec la classification de l’OMS.

Entre 1985 et 2000, 5439 nouveaux patients atteints par la lèpre, encore non traités, furent enregistrés au Centre Schieffelin d’éducation et de Recherche sur la Lèpre de Karigiri. Ils furent classés selon la classification de Ridley et Jopling ainsi que selon la classification opérationnelle de l’OMS qui est basée sur le nombre de lésions.

La sensibilité et la spécificité de la classification pratique de l’OMS, testées en utilisant l’examen bactérioscopique de frottis de suc dermique, ont été déterminées être 88,6% et 86,7%, respectivement. La courbe Caractéristique Opérateur Receveur (ROC) a confirmé que la meilleure option pour la sensibilité et la spécificité est une limite de 6 lésions et plus pour la lèpre multibacillaire (MB).

La validité du nombre de nerfs ayant une taille augmentée ou bien de la taille de la plus grande lésion cutanée comme critère indépendant de classification des patients est faible. L’addition de trois troncs nerveux de taille augmentée à la classification de l’OMS a amélioré sa sensibilité à 91,4%, tandis que la spécificité est restée presque inchangée à 85,3%. L’addition de la taille de la plus grande lésion cutanée à la classification de l’OMS a réduit considérablement sa validité.

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The classification of leprosy determines the type and duration of treatment a patient receives. The World Health Organization (WHO) Study Group on the Chemotherapy of Leprosy in 1993 recommended that clinical criteria might be required for classification of the disease where facilities for the bacteriological examination of skin smears are either unreliable or unavailable (12). The WHO Expert Committee concluded that patients could be classified according to the number of skin lesions into three groups namely, paucibacillary single lesion (PB), paucibacillary leprosy (PB) (2 to 5 skin lesions), multi bacillary leprosy (MB) (6 or more skin lesions) (11).

Several studies have been published on the validity of the clinical criteria, such as number of skin lesions or body area affected, using skin smears and/or histopathological diagnoses as the gold standard. Although the criteria for classification (both clinical and bacteriological) vary among the studies, the sensitivity of the clinical classification ranged from 85% to 93% and specificity from 39% to 88% (2, 3, 4, 7, 9). There is a concern however, that by classifying patients solely on the number of skin lesions, a small but significant number of MB patients may wrongly receive PB treatment and a fairly large number of PB patients would be treated unnecessarily with the MB regimen (4). Therefore, there is good reason to identify and test other simple clinical signs that would complement and improve the validity of the present clinical classification. This paper reviews the validity of the WHO operational classification, and explores whether additional clinical signs that are simple and could be applied in the field would improve the validity of this classification using skin smear examination as the gold standard.

MATERIALS AND METHODS
The Schieffelin Leprosy Research and Training Center (SLR & TC) Karigiri, has been implementing the National Leprosy Eradication Program (NLEP) in the Gudiyatham Taluk since 1962 (Pop. in 1961: 400,000). Leprosy services to the area were provided through mobile clinics to different villages in the control area, supported by leprosy paramedical workers and non-
medical supervisors. All persons with symptoms and signs of leprosy were referred to a medical officer who made the diagnosis based on the presence of the cardinal signs. Patients were classified based on clinical features that would correlate with the six groups of the Ridley Jopling classification (8). Subsequently, they were also classified into MB and PB based on the number of skin lesions as recommended by WHO (11). Patients had a body chart that had a record of the number, distribution, the size of the largest skin lesion, and the peripheral nerves that were enlarged.

Slit-skin smears were part of the routine diagnostic procedure. Standardized methods were used for the preparation, staining, reading, and grading of smears throughout the reference period. Skin and/or nerve biopsies were done only for confirmation of diagnosis or for research purposes. A review of all new previously untreated leprosy patients registered from the control area from 1985 to 2000 was used for this study. The average Bacteriological Index (BI) is used as the gold standard. The sensitivity, specificity, and predictive values were calculated using standard formulae (1). The Receiver Operator Characteristic (ROC) curve is drawn by plotting the sensitivity on the Y axis and 1-specificity or false positives on the X axis. The point on the curve, which is nearest to 100% sensitivity, determines the best cut off point.

RESULTS

A total of 5439 new untreated leprosy patients were registered between 1985 and 2000. Information on the number of skin lesions and skin smear results was available only for 5165 (95.0%) patients. Those with numerous skin patches were included in the group of more than 9 lesions. Persons with no skin lesions, but having other signs of multibacillary leprosy, such as infiltration of the skin, thickened earlobes or nodules, were also included in this group.

Table 1 shows that the sensitivity of the current WHO operational classification that classifies a patient with 6 and more skin lesions as MB is 88.6%, while the specificity is 86.7%. The positive predictive value (PPV) is 39.1% and the negative predictive value is 98.7%. The Receiver Operator Characteristic (ROC) curve (The Figure) illustrates that the best option is at the cut off for 6 and more lesions for classifying a patient as MB (which is the point nearest to 100% sensitivity).

There were 52 smear positive patients who presented with 1 to 5 skin lesions and would have wrongly been classified as PB according to the WHO classification, a false negativity rate of 11.4%. On further analysis, of these 52 patients, 7 (13.5%) had a high BI of 3.00+ or more. Among the 4710 patients who were skin smear negative (Gold Standard), 626 patients were found to have 6 and more skin lesions and hence would have been classified as MB, a false positivity rate of 13.3% (Table 1).

### Table 1. Validity of the WHO operational classification using skin smears as the gold standard.

<table>
<thead>
<tr>
<th>No. of skin lesions</th>
<th>Slit skin smear examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POS</td>
</tr>
<tr>
<td>&gt;5 lesions</td>
<td>403</td>
</tr>
<tr>
<td>1–5 lesions</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>455</td>
</tr>
</tbody>
</table>

Sensitivity: 403 / 455 × 100 = 88.6%
Specificity: 4084 / 4710 × 100 = 86.7%
False positive rate: 626 / 4710 × 100 = 13.3%
False Negative rate: 52 / 455 × 100 = 11.4%
Positive Predictive value: 403 / 1029 × 100 = 39.1%
Negative Predictive value: 4084 / 4136 × 100 = 98.7%

The Figure. The points on the ROC Curve represent sensitivity versus 1–specificity for different cut off points (in terms of the number of skin lesions) used for determining MB cases; the points plotted represent 8 different cut off points ranging from 2 to 9 skin lesions. The point on the curve closest to the point where sensitivity is 100% and 1–specificity is 0 determines the best cut off point, which in this case is 6 lesions.
The validity of independent additional signs such as size of the largest skin lesion and the number of enlarged trunk nerves to classify leprosy was tested. The findings are summarized in Table 2. The number of skin lesions, the size of the largest lesion and the skin smear results were available for 2290 patients. Using the size of the largest skin lesion of 5 centimeters (cm) or more as an independent criterion for classification as MB, the sensitivity was found to be fairly low at 52.9% and a specificity of 71.7%, the sensitivity decreasing further as the size of the lesion increased (Table 2). On combining the size of the largest skin lesion (5 cm or more as MB) to the WHO operational classification, the sensitivity improved only slightly to 58.8%, while the specificity remained almost the same at 70.4%. When the size of the largest skin lesion is more than 8 cm, the sensitivity decreased considerably to 35.3% while the specificity increased to 86.4% (Table 3).

The validity of using the number of enlarged trunk nerves as an independent criterion for classification as MB was tested (Table 2). The number of skin lesions, the number of enlarged nerves and the skin smear results were available for 3650 patients. The sensitivity was found to be 81.0% and the specificity was 79.9% for one or more enlarged nerves. It is observed that the sensitivity steadily decreases as the number of enlarged nerves increased, while on the other hand, the specificity increased considerably to 91.8% when two or more nerves are enlarged, further increasing to 99.2% when five or more nerves are enlarged.

On adding the number of enlarged nerves to the WHO operational classification, a good balance of sensitivity and specificity was observed when enlargement of three nerves or more were added to the WHO classification, with a sensitivity of 91.4% and a specificity of 85.3%. This gives a fairly low false positivity rate of 8.6%, but a false negative rate of 14.7%. The positive predictive value is 39.5% and the negative predictive value is 98.9% (Table 4).

**DISCUSSION**

Using skin smear examination as the gold standard has its limitations. It has been reported that using skin smear alone may not be as sensitive as using skin smears in combination with skin and/or nerve biopsies (4, 7). The reliability of skin smears has been reported to be low (6, 10). However, at laboratories with good standardization of techniques

---

**Table 2. Validity of using size of largest skin lesion or enlarged trunk nerves as independent criteria using skin smear as the gold standard.**

<table>
<thead>
<tr>
<th>Clinical criteria</th>
<th>Total no.</th>
<th>SS + ve</th>
<th>SS – ve</th>
<th>SS + ve</th>
<th>SS – ve</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>with</td>
<td>with</td>
<td>with</td>
<td>with</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>positive</td>
<td>positive</td>
<td>negative</td>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Largest skin lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5 cms</td>
<td>652</td>
<td>9</td>
<td>643</td>
<td>8</td>
<td>1630</td>
<td>52.9</td>
<td>71.7</td>
</tr>
<tr>
<td>≥ 8 cms</td>
<td>267</td>
<td>4</td>
<td>263</td>
<td>13</td>
<td>2010</td>
<td>23.5</td>
<td>88.4</td>
</tr>
<tr>
<td>No. of enlarged trunk nerves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 nerve</td>
<td>946</td>
<td>282</td>
<td>664</td>
<td>66</td>
<td>2638</td>
<td>81.0</td>
<td>79.9</td>
</tr>
<tr>
<td>2 nerves</td>
<td>511</td>
<td>240</td>
<td>271</td>
<td>108</td>
<td>3031</td>
<td>68.9</td>
<td>91.8</td>
</tr>
<tr>
<td>5 nerves</td>
<td>70</td>
<td>46</td>
<td>24</td>
<td>302</td>
<td>3278</td>
<td>13.2</td>
<td>99.2</td>
</tr>
</tbody>
</table>

* SS = Skin smear  TP—True positives  FP—False positives  TN—True negatives  FN—False negatives

**Table 3. Validity of adding size of the largest skin lesion to the WHO operational classification standard.**

<table>
<thead>
<tr>
<th>More than 5 skin lesions and size of largest lesion (cms) among those with 1–5 skin lesions</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;2 cm</td>
<td>82.4</td>
<td>24.5</td>
</tr>
<tr>
<td>&gt;5 cm</td>
<td>58.8</td>
<td>70.4</td>
</tr>
<tr>
<td>&gt;8 cm</td>
<td>35.3</td>
<td>86.4</td>
</tr>
<tr>
<td>&gt;10 cms</td>
<td>29.4</td>
<td>91.2</td>
</tr>
</tbody>
</table>
and quality control, skin smears could be considered to have a fairly high reliability.

Two important points have to be considered while discussing validity of test criteria such as classification based on clinical criteria into MB/PB leprosy. One is the ability of the criteria to correctly identify the MB patients (true positives or sensitivity) and thereby minimize the number who would be wrongly classified as PB, while in fact they are MB patients (false negatives). Wrongly classifying a MB patient as PB is a cause for concern due to the increased risk of relapse and reactions in MB patients, as well as the inadequate treatment they would receive.

The second issue is the ability of the criteria to be able to correctly identify those who are not MB (true negatives or specificity) and consequently minimize the number of PB patients who would be diagnosed as MB (false positives). Wrongly classifying a MB patient as PB is a cause for concern due to the increased risk of relapse and reactions in MB patients, as well as the inadequate treatment they would receive.

The second issue is the ability of the criteria to be able to correctly identify those who are not MB (true negatives or specificity) and consequently minimize the number of PB patients who would be diagnosed as MB (false positives). Wrongly classifying a MB patient as PB is a cause for concern due to the increased risk of relapse and reactions in MB patients, as well as the inadequate treatment they would receive.

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The second issue is the ability of the criteria to be able to correctly identify those who are not MB (true negatives or specificity) and consequently minimize the number of PB patients who would be diagnosed as MB (false positives). Wrongly classifying a MB patient as PB is a cause for concern due to the increased risk of relapse and reactions in MB patients, as well as the inadequate treatment they would receive.

TABLE 4.  Validity of adding the number of enlarged nerves to the WHO operational classification.

<table>
<thead>
<tr>
<th>More than 5 skin lesions and no. of nerves involved among those with 1 to 5 skin lesions</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>93.4</td>
<td>74.9</td>
</tr>
<tr>
<td>2</td>
<td>92.5</td>
<td>83.0</td>
</tr>
<tr>
<td>3</td>
<td>91.4</td>
<td>85.3</td>
</tr>
<tr>
<td>4</td>
<td>90.2</td>
<td>85.7</td>
</tr>
<tr>
<td>5</td>
<td>89.1</td>
<td>86.0</td>
</tr>
</tbody>
</table>

With the addition of more than two enlarged nerve trunks to the WHO operational classification increased the sensitivity to 91.4% without altering the specificity much (85.3%). Consequently, the number of false negatives and false positives are fairly low, bringing down the number of MB patients who would be under treated.

Considering that nerve enlargement is one of the cardinal signs for diagnosis of leprosy, its inclusion in the classification should not be very difficult. However, application under field conditions for use by general health workers might be difficult, since identifying nerve enlargement per se is subjective, with a fairly high inter and intra observer variation. The WHO recommended that a patient with enlargement of more than one nerve trunk could be classified as MB (10). It was observed that the sensitivity of using such a criterion independently was 68.9%, which is rather low, while the specificity is fairly high at 91.8%.

The addition of the size of the largest skin lesion to the WHO operational classification showed considerable decrease in the sensitivity and the specificity. However, it should be noted that the among the smear positive patients, only a small proportion had a record of the size of the largest skin lesion (4.9%). Since the measurement of the largest skin lesion is fairly simple and could be applied in the field, it may be worthwhile to recheck its validity using another data set.

The classification of leprosy using clinical criteria has its benefits and limitations. While it is easy and simple to apply in the field by general health workers, there is always the risk of a certain proportion of patients being under or over treated. Based on the WHO operational classification this study found that about 11% of the patients would be under-treated, while 13% would be overtreated. The validity of using number of enlarged trunk nerves and the size of the largest skin lesion as independent criteria was low. Addition of enlarged trunk nerves to the operational classification improved its sensitivity with no significant change to the specificity; on the contrary, addition of the size of the largest skin lesion to the operational classification lowered its validity considerably.

It appears from this study, that the WHO recommendation of using six lesions for classifying a patient as MB is the best option available at the moment and calls for further research to identify other clinical criteria that have a better validity and can be applied in the field.

Acknowledgment. The authors wish to thank Mr. P. Samuel, Leprosy Supervisor, Mr. Raja Samuel, Programmer, and Mr. P. Dorairaj Medical Records Technician for all the help rendered in the collation and analysis of the data. The authors appreciate the Department of Laboratories and the smear technicians for their efforts in maintaining high standards in examination of skin smears.
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Pure Neuritic Leprosy in India: an Appraisal

Bhushan Kumar, Inderjeet Kaur, Sunil Dogra, and Muthu Sendhil Kumaran

ABSTRACT

Background: Pure neuritic leprosy (PNL) constitutes a significant proportion of all cases in India, however, this form of disease has not been fully recognized and investigated and there is little information in the existing literature.

Objective: To study the epidemiological characteristics of PNL in India.

Materials and Methods: A retrospective analysis of leprosy clinic records for the period 1993 to 2003 was undertaken. Detailed demographic profiles and clinical findings were noted from the predesigned clinic proforma. A slit-skin smear for acid-fast bacilli (AFB) was done in all cases from the area of sensory loss. A skin biopsy was done from the area of sensory impairment to study histopathological changes. Further investigations such as nerve conduction velocity studies (NCV), fine needle aspiration cytology (FNAC), or nerve biopsy (superficial nerve twigs) were done if indicated in patients whenever there was difficulty in clinical diagnosis.

Results: Of the total 1542 leprosy patients seen over this period, 65 (4.2%) had PNL. Males were more commonly affected than females (2.6:1.). The majority of patients 40/65 (61.5%) were aged between 15 and 35 yrs. Predominant presenting symptoms were paresthesia, pain, sensory/motor deficit, and trophic changes. A majority of the patients 39/65 (60.0%) presented with involvement of 2 or more nerves in the same extremity. Mononeuritis was seen in 26 (40%) patients. The nerves most often involved were the right ulnar nerve in the upper extremity, and the right common peroneal nerve in the lower limb. In general, the nerves of the upper extremity were more commonly involved than in the lower limbs (67 vs. 55). Motor deformities such as claw hand and foot drop were present in 13/75 (20%) and 7/65 (10.8%) patients, respectively. Slit-skin smears were negative in all patients, and skin histopathology from the area of sensory loss revealed non-specific inflammation in the dermis in a majority of patients, with perineural inflammation in a few. All patients were treated with multi-drug therapy (MDT); patients with ≥2 peripheral nerve trunk involvements were treated with WHO MDT MB regimen, while others were administered WHO MDT PB regimen. Follow-up for up to 2 yrs was available in only 32/65 (49.2%) patients, none of whom developed any skin lesions during this period.

Conclusion: PNL is a distinct subset of disease frequently seen in India. There is need to pay more attention to this form of leprosy and diagnose and treat patients earlier to prevent deformities and sequelae of nerve damage.

RÉSUMÉ

Background: La lèpre névritique pure (LNP) représente une proportion significative du total des cas observés en Inde. Cependant, cette forme de la maladie n’a pas été complètement caractérisée et reste peu identifiée, avec peu d’information dans la littérature.

Objectif: Étudier les caractéristiques épidémiologiques de la LNP observée en Inde.

Matériel et méthode: Une analyse rétrospective des dossiers cliniques de patients lépreux de l’intervalle 1993–2003 fut entreprise, comportant une extraction détaillée des profils démographiques et des signes cliniques à partir de données présentes dans des fiches cliniques pré-établies standardisées. Un examen bactérioscopique de recherche de bacilles acido-résistants (AAR) fut effectué dans tous les cas, à partir de frotis de sue derrique provenant des aires de perte sensorielle. Une biopsie cutanée fut réalisée à partir des aires de perte sensorielle afin d’étudier les changements histopathologiques. Des investigations complémentaires, telles que des études de vitesse de conduction nerveuse (VCN), des aspirations à l’aiguille fine (AAF) en vue d’exams cytologiques, ou des biopsies...
nerveuses (rameaux nerveux superficiels), furent mises en œuvre dès que des difficultés de diagnostic clinique le justifiaient chez certains patients.

Résultats: Soixante cinq parmi 1542 patients lépreux (4,2%) présentaient une LNP. Les hommes étaient plus communément affectés que les femmes (2,6:1). La majorité (40/65 ou 61,5%) était âgée de 15 à 35 ans. Les symptômes prédominants à l’examen initial étaient des paresthésies, des douleurs, des déficits moteurs et/ou sensoriels et des altérations trophiques. La majorité des patients (39/65 soit 60,0%) présentaient une atteinte de 2 nerfs ou plus invariant le même membre. Les mono-névrites ne furent observées que chez 26 (40,0%) des patients. Les nerfs les plus communément atteints étaient le nerf ulnaire droit pour les membres antérieurs et le nerf péroné commun pour les membres intérieurs. En général, les nerfs des membres antérieurs étaient plus communément atteints que ceux des membres postérieurs (67 versus 55). Les difformités d’origine motrices, telles que les mains en crochet et les pieds tombants, ont été observées chez 13/65 (20%) et 7/65 (10,8%) des patients, respectivement. L’examen bactérioscopique à partir de suc dermique fut négatif chez tous les patients atteints de LNP, et l’examen histologique de la peau provenant de zones avec perte sensorielle n’a révélé qu’une inflammation non spécifique du derme chez la majorité des patients, avec une inflammation péri-nerveuse dans quelques cas. Tous ces patients furent traités par une poly-chimiothérapie (PCT) ; les patients présentant une atteinte de 2 nerfs ou plus furent soignés par le traitement recommandé par l’OMS concernant les patients multibacillaires, tandis que les autres reçurent le traitement des patients paucibacillaires. Un suivi ne dépassant pas 2 années n’était présent que chez seulement 49/65 (49,2%) des patients. Ces derniers n’ont pas développé de lésions cutanées durant cette période.

Conclusion: La LNP est une catégorie distincte de la lèpre qui est assez fréquemment observée en Inde. Il va être nécessaire de prêter une plus grande attention à cette forme de la maladie afin de diagnostiquer et traiter les patients plus précocement, ce qui devrait permettre de prévenir les difformités et séquelles secondaires aux lésions nerveuses.

RESUMEN

Panorama: La lepra neurítica pura (LNP) constituye una significante proporción de todos los casos de lepra en la India, sin embargo esta forma de la enfermedad no se ha estudiado con el detalle requerido y por esto casi no hay información al respecto en la literatura.

Objetivo: Estudiar las características epidemiológicas de la LNP en la India.

Materiales y Métodos: Se realizó un análisis retrospectivo de la lepra en los archivos clínicos correspondientes al periodo de 1993 a 2003. Los perfiles demográficos detallados y los hallazgos clínicos fueron concentrados en un formato prediseñado. En todos los casos se buscaron bacilos ácido resistentes en la linfa cutánea de las regiones con pérdida sensorial y también de estas regiones se tomaron biopsias para su estudio histopatológico. En los pacientes con diagnóstico clínico impreciso se hicieron además otros estudios tales como medición de la velocidad de conducción nerviosa (VCN), citología por aspiración con aguja fina (CAAF), o biopsia de los nervios superficiales.

Resultados: Del total de 1542 pacientes con lepra vistos en este periodo, 65 (4.2%) tuvieron PNL. Los hombres fueron más afectados que las mujeres (2.6:1). La mayoría de los pacientes 40/65 (61,5%) tuvieron entre 15 y 35 años. Los síntomas más aparentes fueron parestesia, dolor, déficit sensorial/motor, y cambios tróficos. La mayoría de los pacientes 39/65 (60%) presentaron involución de 2 ó más nervios en la misma extremidad. Veintiséis pacientes (40%) tuvieron mononeuritis. Los nervios más frecuentemente afectados fueron el ulnar derecho en la extremidad superior y el peroneal común derecho en la extremidad inferior. En general, los nervios de la extremidad superior fueron más frecuentemente afectados que los nervios de la extremidad inferior (67 vs 55). Las deformidades motoras tales como mano en garra y pie caído estuvieron presentes en 13/75 (20%) y 7/65 (10.8%) pacientes, respectivamente. Los extendidos de linfa cutánea fueron negativos en todos los pacientes y la histopatología del área anestésica no reveló inflamación inespecífica en la dermis en la mayoría de los pacientes, aunque algunos presentaron inflamación perineural moderada. Todos los pacientes fueron tratados con poliquimioterapia (PQT); los pacientes con afectación de dos o más troncos nerviosos periféricos fueron tratados con el régimen de PQT de la OMS para la lepra multibacilar. El seguimiento por 2 años sólo se pudo hacer en 32/65 (49,2%) pacientes, ninguno de los cuales desarrolló ninguna lesión en la piel durante este periodo.

Conclusión: La LNP es un tipo particular de la enfermedad frecuentemente observado en la India. Es necesario poner más atención a esta forma de la lepra con objeto de diagnosticar y tratar a los pacientes de manera temprana para prevenir las deformaciones y secuelas del daño a nervios.
Nerve damage and the consequences of nerve damage set leprosy apart from other diseases. Leprosy would be a rather innocent skin disease were it not for the nerve damage and subsequent deformities, which make the leprosy patient an outcast from society. Considering the central place that nerve damage occupies in leprosy and in the personal and social consequences of the disease, it is perhaps surprising how little importance its recognition and treatment is given in most elimination campaigns, where the emphasis is very much on MDT only \(^1\). Nerve involvement, whether clinical or microscopic, is fundamental to the pathogenesis of leprosy, and it should receive more attention in the elimination program to reduce the burden of deformities in the community. Although both skin and nerve involvement usually occur together in leprosy, there can also be nerve involvement without any primary skin lesion. This form of disease has been called neuritic leprosy (pure neural, primary neural, pure neuritic, primary neuritic, polyneuritic) \(^2\). Although such patients constitute a significant proportion of all cases, especially in India, this category of patients has not been fully recognized or thoroughly investigated, with scarce published information in the literature \(^3, 10, 12, 15, 16, 20, 26, 27\).

The Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, is a tertiary care center in Northern India, which is a low endemic area for leprosy. In addition to the population of this region, the Institute also caters to a large migrant population from various states of the country where leprosy is endemic. Systematic analysis of the leprosy clinic records at our center was carried out to study various epidemiological characteristics of patients with pure neuritic leprosy (PNL) over a period of last 10 yrs.

**MATERIALS AND METHODS**

Clinic records of patients diagnosed as PNL enrolled at our center from 1993 to 2003 were analyzed. PNL was defined as thickened peripheral nerve trunk with sensory loss in the area of its distribution, with or without associated motor paralysis in the absence of any skin patch regardless of clinical evidence of reaction involving nerve(s) \(^2\). Detailed demographic profile and clinical findings were recorded in the predesigned clinic proforma. Slit-skin smear for acid-fast bacilli (AFB) was done in all cases from the area of sensory loss. It was ensured by at least two observers that we were not missing any skin lesions. A skin biopsy was done from the area of sensory impairment to study histopathological changes. Further investigations like nerve conduction velocity studies (NCV), fine needle aspiration cytology (FNAC), or nerve biopsy (superficial nerve twigs) were done if indicated in patients whenever there was difficulty in clinical diagnosis. All patients were treated with multi-drug therapy (MDT). Patients with \(\geq 2\) peripheral nerve trunk involvements were treated with WHO MDT MB regimen while others were administered WHO MDT PB regimen. All patients with recent onset of motor deficit within the preceding year were treated with a 6 month course of oral prednisolone in tapering doses. All reaction episodes were managed with oral corticosteroids given over 12 to 16 week period. Patients were instructed to report for follow-up twice a year for at least two years after completing therapy or immediately in case of any symptom suggestive of acute neurological deficit. The objective of the present study was to analyze epidemiological features of PNL such as their frequency, age and sex distribution, presenting symptoms, number of nerves involved, common sites of nerve involvement, deformities, and evidence of reactions etc.

**RESULT**

Of the total 1542 leprosy patients seen over this period, 65 (4.2%) had PNL. In 9 patients with nerve thickening but equivocal neurological deficit, the diagnosis was confirmed by NCV studies. In addition, in 2 patients it was supported by FNAC and nerve biopsies. The age and sex distribution of patients with PNL is given in Table 1. Males were more commonly affected than females \((2.6:1)\). The majority of the patients \(40/65 (61.5\%)\) were aged between 15 and 35 yrs. Most of the patients with PNL presented to us with more than one symptom. Predominant presenting symptoms were paresthesia, pain, sensory/motor deficit, and trophic changes (Table 2). A majority of the patients \(39/65 (60.0\%)\) pre-
sented with involvement of 2 or more nerves in the same extremity. Mononeuritis was seen in 26 (40%) patients. Involvement of more than one limb was not uncommon, and up to 7 nerves were involved in 6 (9.2%) of the 65 patients. The nerves most often involved were right ulnar nerve in the upper extremity, and the right common peroneal (lateral popliteal) nerve in the lower limb. In general, the nerves of the upper extremity were more commonly involved than those in the lower limbs (67 vs. 55) (Table 3). Slit-skin smears from all sites including the area of anesthesia were negative for AFB in all the patients. Skin histopathology revealed non-specific inflammation in the dermis in a majority of patients with perineural inflammation in a few. However, no well-formed granuloma or AFB were discernable in any skin biopsy specimen.

Twenty patients had symptoms suggestive of neuritis in the form of pain and paresthesia along the distribution of affected nerve trunk. Motor deformities such as claw hand and foot drop were present in 13/75 (20%) and 7/65 (10.8%) patients, respectively. Of these 13 patients, 11 had partial (ulnar) and 2 had complete (ulnar + median) claw hand. Various trophic changes in the form of trophic ulcers, xerosis, fissured hands and soles were seen in 10 (15.4%) patients. More patients had planter ulcers, 8/10 (80%), than those over hands, 2/10 (20%); 5 of them had more than one trophic ulcer.

Sixteen (24.6%) patients had motor deficit of recent onset, and treatment with oral prednisolone resulted in significant improvement in 6 months. All patients 20/65 (30.8%), who had neuritis [painful tender nerve(s) with paresthesia or sudden increase in anesthesia] at the time of presentation, and 4/65 (21.5%) patients who developed it during treatment with MDT responded well to a course of systemic steroids. Two patients (3%) had an abscess in the ulnar nerve, which was managed by surgical drainage along with MDT and prednisolone.

Only one of our patients (on MDT PBR) developed multiple skin lesions during the treatment, and responded well to MDT MBR. Follow-up of up to 2 yrs was available in only 32/65 (49.2%) patients, none of whom developed any skin lesions during this period.

**DISCUSSION**

Although its overall prevalence is decreasing, leprosy continues to be a major cause of neuropathy and a significant cause of disability worldwide (14). PNL is a well-recognized clinical entity in India (2, 15). It is characterized by neural signs manifested by an area of sensory loss along the distribution of a thickened nerve trunk with or without motor deficit and absence of any skin patch (15). Experimental evidence has suggested that *Mycobacterium leprae* can

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**Table 1. Demographic parameters of patients.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex M/F</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤14</td>
<td>4/0</td>
<td>4</td>
</tr>
<tr>
<td>15–25</td>
<td>15/6</td>
<td>21</td>
</tr>
<tr>
<td>26–35</td>
<td>12/7</td>
<td>19</td>
</tr>
<tr>
<td>36–45</td>
<td>7/2</td>
<td>9</td>
</tr>
<tr>
<td>46–55</td>
<td>5/3</td>
<td>8</td>
</tr>
<tr>
<td>≥56</td>
<td>4/0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>47/18</td>
<td>65</td>
</tr>
</tbody>
</table>

**Table 2. Presenting symptoms. (Many patients presented with more than one symptom.)**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paresthesia</td>
<td>10</td>
</tr>
<tr>
<td>Pain in the area supplied by the nerve</td>
<td>20</td>
</tr>
<tr>
<td>Sensory deficit</td>
<td>43</td>
</tr>
<tr>
<td>Motor deficit</td>
<td>20</td>
</tr>
<tr>
<td>Trophic changes</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 3. Sites of nerve involvement.**

<table>
<thead>
<tr>
<th>Nerves involved</th>
<th>Number and location of nerves involved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
</tr>
<tr>
<td>Ulnar</td>
<td>30</td>
</tr>
<tr>
<td>Median</td>
<td>2</td>
</tr>
<tr>
<td>Radial</td>
<td>0</td>
</tr>
<tr>
<td>Radial cutaneous</td>
<td>8</td>
</tr>
<tr>
<td>Common peroneal</td>
<td>16</td>
</tr>
<tr>
<td>Posterior tibial</td>
<td>12</td>
</tr>
<tr>
<td>Sural</td>
<td>4</td>
</tr>
<tr>
<td>Greater auricular</td>
<td>3</td>
</tr>
</tbody>
</table>
gain entry through skin (9) as well as through upper respiratory tract (1). Talwar, et al. (26) proposed that entry through the skin produces mononeuritic type of disease, while the respiratory route may be responsible for the polyneuritic type of disease. It is postulated that after entering through the skin, *M. leprae* invades axoplasmic filaments and only after rupturing Schwann sheath the organism can burst into the corium of the skin (2). PNL may be an early stage in the pathogenesis of the disease before the appearance of skin lesions. Roche, et al. (19) investigated the serological response to two *M. leprae* specific epitopes (PGL-1 and 35K Da protein) in 46 untreated patients with PNL. They reported a significant increase in the level of antibody response (IgM) when more nerve trunks were involved. Changes in antibody levels in seropositive PNL patients may prove useful in monitoring the response to chemotherapy.

The occurrence of PNL shows marked regional variations. PNL is frequently seen in India, while it is much less common in some other countries. According to Noordeen (15), PNL contributes up to one-sixth of all leprosy cases in India. Frequency of its occurrence varied from 4.6% (12) to 5.5% (1) in western India to 17.7% in South India (15). Some cases may be misdiagnosed, so available figures may be lower than the actual proportion of cases in the community. In our study (North India), PNL accounted for 4.2% patients. In most of the earlier studies (12, 15, 16), the majority of cases were aged above 40 yrs. Noordeen (15) observed steadily rising incidence with increasing age. In contrast to previous reports, a majority of patients in our study were between 15 and 35 yrs of age. This could be attributed to an increased level of awareness about the disease in the general population and relatively early detection of cases because of elimination campaigns in the last decade, or to regional variation in the epidemiology of disease. As observed earlier (12, 15, 16), this form of leprosy is relatively uncommon in children, in our study children constituted only 6.1% of the total patients. Like us, some previous workers (10, 15, 27) also observed male preponderance in PNL as is present even in other types of leprosy.

Mononeuritis has been the most common presentation in some of the previous studies (12, 15, 27), where as in our study, involvement of two nerves of the same extremity was the most frequent finding (60%) and mononeuritis was seen in 40% of our patients. Involvement of multiple nerves at the time of presentation has also been highlighted by some other workers (12, 16). As observed by us, a majority of the previous reports (12, 16, 26, 27) have also noted ulnar nerve to be the commonest affected nerve in PNL. However, Noordeen (15) reported the lateral popliteal nerve to be most commonly affected in PNL.

Pure neuritic cases are probably more vulnerable to nerve damage as supported by a high frequency of deformities reported by various workers (10, 12, 26). This could be because of more delay in diagnosis of PNL patients than those with skin lesions. So it is important to recognize the symptoms early. Unfortunately patients usually ignore or misinterpret the symptoms and present rather late, after significant nerve damage has already occurred; 20 (30.7%) of our patients presented with a predominant motor deficit, which is a rather late occurrence. Deformities in leprosy could be secondary to nerve involvement in untreated disease or because of reactions. Though grading of deformities may vary at different centers, a high deformity rate in PNL has been reported in many studies [Talwar, et al. 16.1% (26), Kaur, et al. 31.5% (10), Mahajan, et al. 48.6% (12)]. Reactions in the form of neuritis at the time of presentation or during the treatment were seen in 34/65 (52.3%) of our patients. No definite figures on reactions are available in other series on PNL. Nerve abscess, when present, makes clinical diagnosis of PNL easy. Though there are isolated case reports (21), the frequency of its occurrence in other large series on PNL has not been reported.

Diagnosis of PNL can be mostly made on clinical examination in a majority of the cases, especially in endemic areas (2, 8, 23). Although nerve biopsy is ideal for diagnosis, it is often not practicable and has its own limitations (7, 13). Recently polymerase chain reaction (PCR), FNAC and various electrophysiologic studies have been reported to be useful laboratory adjunct in confirming diagnosis of doubtful cases (8, 18, 28). However,
the expertise for the technique and sensitivity of this test are the limitations. The importance of a biopsy from normal looking anaesthetic skin is to exclude the possibility a cutaneous leprosy lesion which may not be clinically apparent, although histopathologically the evidence can be convincing (3). Findings in the apparently normal skin and nasal mucosa in patients with the diagnosis of PNL indicate that there are widespread changes due to leprosy even when the disease appears clinically confined only to a few nerves (24, 25). Suneetha, et al. (24) reported histological changes of leprosy in the apparently normal looking skin of 32.1% patients of PNL, with 12.7% of them having either epithelioid or macrophage granulomas. They proposed that absence of visible hypopigmented skin lesions in these patients is probably related to the deep location of the granuloma in the dermis, beyond the scope of exercising any direct influence on the melanocytes in the epidermis. However, similar to our observations, Kaur, et al. (10) did not find any significant pathology in the anaesthetic skin of patients with PNL.

There are no defined WHO guidelines about the classification of these cases and therefore, about treatment (6). Many of these cases are simply considered to belong to the PB group since all of them are SSS negative and are mostly lepromin positive (2). However, nerve biopsy in several cases has revealed features of borderline or even lepromatous spectrum with large number of bacilli (10,16). Unlike skin lesions, it is not possible to routinely carry out invasive investigations (biopsy, smear, etc.) in patients with PNL to classify the disease for treatment purposes. The number of nerve trunks involved can be taken as a reliable clinical criterion for determining the MDT regimen (PB/MB).

The long-term course of PNL is not fully clear. Noordeen, in his field observations from South India, reported that there is a tendency for spontaneous resolution of some cases of PNL (15). However, this observation has not been made by others. Subsequent development of skin lesions (most commonly, borderline tuberculoid) is well known, with many case reports in the published literature (15, 16, 17, 22, 24, 27). In a study by Talwar, et al. (26), 25% and 7% of their PNL patients treated with dapsone mono-therapy and MDT PBR developed skin lesions at an average period of 3 and 2.6 months, respectively. In a study from South India (24), 15.9% patients with PNL developed skin lesions during follow up period varying from 6 months to 12 yrs. However, Mahajan, et al. (12) did not report skin lesions in any of their patients followed up to 18 months after stopping MDT. It has been suggested that this phenomenon is more likely in those patients of PNL, who are not treated adequately or when treatment is irregular, whereas, prompt and better killing of the organisms by adequate MDT arrests the spread of disease from the nerves to skin preventing development of skin lesions (26). Only one of our patients (on MDT PBR) developed skin lesions during the course of anti-leprosy treatment, and was later treated with MDT MBR. In addition, the possibility of developing new skin lesions during treatment as part of a reversal reaction should be kept in mind (4).

Although a lot has been achieved towards eliminating leprosy, there is yet a long way to go. We should ensure that during this transition from dedicated leprosy programs to their amalgamation with general health services, adequate awareness and expertise about the timely diagnosis and treatment of this neural phenomenon is maintained, as such cases will probably continue to occur sporadically in the “post-elimination era.”

REFERENCES


Deep pain upon percussion of lesions over bone in tuberculoid leprosy, in spite of superficial sensory impairment, has been described as the “Tap Sign” (TS). This study was conducted to identify possible causes for this phenomenon and to determine the sensitivity and specificity of this sign in leprosy patients with lesions overlying bone. In 37/53 patients with lesions over bone, the sensitivity of the TS was 66.7% and the specificity was 100%. The positive predictive value was 100%, and the negative predictive value was 75%. The Tap Sign appears to be a useful clinical sign in diagnosis of tuberculoid and borderline tuberculoid leprosy where a lesion overlies a bone (sensitivity 66.7%). This test could be very useful to increase the clinical diagnostic yield, in the global perspective, in places where leprosy is diagnosed and treated by healthcare workers and primary care physicians without other laboratory facilities. Possible mechanisms responsible for the deep pain are discussed.
Most researchers classify leprosy into 5 main types: polar lepromatous (LL), borderline lepromatous (BL), borderline (BB), borderline tuberculoid (BT), and polar tuberculoid (TT) (9). Many patients with tuberculoid (TT) and borderline tuberculoid (BT) leprosy (with semianaesthetic lesions) over bony prominences give a history of “deep pain” when the relevant area is accidentally knocked on a hard object, such as a table top or a chair. A similar sensation can be elicited on tapping the affected area overlying bone, with a paper weight or even with the knuckles of the examining clinician. This paradoxical phenomenon (deep pain, in spite of superficial sensory impairment) was described by the principal author as the “Tap Sign” (Kumarasinghe, 2001) (7). “Tap Sign” (TS) is not usually elicited in polar lepromatous leprosy (LL).

MATERIALS AND METHODS

We conducted a study, at the Dermatology Clinic, Base Hospital Panadura, Sri Lanka, to study possible causes for the TS (Part 1) and to determine the sensitivity and specificity of TS in leprosy patients with lesions overlying bone (Part 2).

In the first part of the study, 11 consecutive patients with TT and BT with a positive TS were recruited. Skin biopsy, fasting blood sugar, blood urea, white cell, count and ESR were done. The sites on which TS was elicited were x-rayed.

In Part 2 of the study (carried out after Part 1 was completed, on additional patients) 53 new leprosy patients were analyzed. Where a lesion of leprosy was visible over a bone (e.g., elbow, radial border), TS was assessed. Figure 1 shows the technique of eliciting the TS with a paperweight (approximate weight 100 gm). The patients’ perception of intensity of pain sensation was compared with the corresponding site on the contralateral side of the same patient. They were requested to say whether pain sensation upon tapping was greater, less, or the same. TS was considered to be positive when the deep pain sensation was greater over the lesional area.

RESULTS

In Part 1 of the study, haematological and biochemical investigations of all patients were normal. The x-rays did not show any periosteal sclerosis, cysts, changes of osteomyelitis, or any other abnormalities. The diagnosis of leprosy was confirmed histopathologically. Histopathology of the TS positive patients did not show any major differences when compared with other biopsies from areas not overlying bone.

In Part 2, the mean age was 34.05 yrs (range 8 to 76 yrs). There were 32 females and 21 males. Of all the leprosy cases, 37/53 (69.8%) had lesions over bone. When all leprosy types were considered 21/37 (56.7%) had a positive TS. Of TT and BT leprosy patients with a lesion over bone, 18/27 (66.7%) had a positive TS on affected side. 14/18 (77.8%) of this cohort were TT and 4/18 (22.2%) were BT. Among multibacillary (MB) leprosy patients (BL, subpolar LL and LL), with lesions over bone, TS was positive only in 3 of 10 (30%) patients. These patients were either BL or subpolar LL patients. None of the polar LL (“pure” lepromatous) patients (N = 5) had a positive TS, although they had lesions overlying bone. In the 2 pure neuritic leprosy patients without skin lesions, TS was negative.

Table 1 shows TS positive and negative groups statistically tabulated. Table 2 shows the measure of association and 95% confidence intervals. The sensitivity of the TS in this study was 66.7% and the specificity was 100% (for lesions overlying bone). The positive predictive value was 100%, and the negative predictive value was 75%.

The size of the skin lesion did not correlate with demonstration of TS or its intensity, as long as the lesion was of sufficient size to test for the TS, e.g., >3 cm. The majority of the patients with a positive TS had had the lesions for less than one year (13/21, 61.9%). The common sites where TS was demonstrated included: olecranon of the elbow, ulnar border, radial border, patella, tibia, proximal digits, and metacarpals (dorsally) and metatarsals (dorsally).

DISCUSSION

Leprosy is still a major public health issue in many parts of the world (3, 6, 10, 11, 12). The incidence of leprosy in Sri Lanka has been below 1/10,000 since 1995, and has been declining in the recent past (9). Leprosy is diagnosed and treated in field clinics...
in many developing countries, by physicians as well as by trained field workers (11, 12). Facilities for skin biopsy and microbiological investigations are not available in remote areas. Additional clues to diagnosis of leprosy are useful, in the global perspective (3). Wrong diagnosis of leprosy may unnecessarily stigmatize the patient and erroneous treatment with multiple drugs may even cause serious side effects, e.g., haemolytic anaemia or agranulocytosis due to dapsone.

The pain elicited by TS is not a radiating pain, but a dull aching “bony” pain which lasts several seconds. This deep pain sensation is different to the neuralgic pain along the distribution of a sensory nerve trunk or “electric current like pain” when a nerve is accidentally knocked (e.g., ulna nerve at the elbow). It is also different from paraesthesia of peripheral neuropathies. Over the area where tap sign is elicited superficial skin sensations are impaired (when tested with cotton wool or nylon bristles) None of the patients recruited in these studies had any reactional states.

In this study, we demonstrated that 66.7% of patients with hypopigmented skin lesions overlying bone had a positive TS, indicating that it is a useful sign to elicit. None of the non-lesional sites tested for comparison showed any positive TS. This indicates that contralateral non-lesional bony prominences are suitable sites for direct comparison.

**Table 1. Comparison of tap sign positive cases, in patients with lesions over bone, with corresponding contralateral sites.**

<table>
<thead>
<tr>
<th>Hypopig. lesion over bone</th>
<th>Contralateral site (non-lesional)</th>
<th>Total tested sites (lesional &amp; non-lesional)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap sign positive</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Tap sign negative</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54</td>
</tr>
</tbody>
</table>
In a subsequent patient, TS was positive over a semianaesthetic area overlying bone, even without any visible evidence of hypopigmentation. The diagnosis in this case was confirmed by biopsy. This suggests that TS can antedate visible cutaneous disease.

We recommend that suspected leprosy patients should be specifically asked for a history of, “deep pain lasting for several seconds when a hypopigmented macule overlying bone is knocked against a hard object.” If there is a positive response by the patient, the suspicion of leprosy should be higher, and this could be tested objectively by eliciting TS.

There are several theories to explain the phenomenon of TS. The authors feel that the first possibility (see below) is the most likely.

(i) Where the sensory input from the overlying skin is diminished or absent, due to cutaneous nerve destruction due to leprosy, the pain sensitive periosteal pain fibers may become “relatively more sensitive” and “perceive” more pain, when knocked or tapped on. Periosteum is a dynamic tissue with a rich sensory nerve supply as well as a sympathetic nerve supply (1, 4). It is known that the periosteum of the bones near the skin receives sensory nerve supply from the skin, whereas the periosteum of deep bones receives innervation via the muscle attachments. Where TS was negative in TT or BT lesions overlying bone, it is possible that the sensory periosteal nerves are also destroyed by the leprosy disease process.

TS phenomenon may be similar to the “increased perceived hearing” on the side of air conduction deficit, compared to the side of normal air conduction, when tested with a tuning fork, in a patient with a defect in air conduction in the ear canal (Weber’s test). Interestingly, when vibration sense of the bone was tested over the TS positive sites (in some patients), there was no difference on the two sides, indicating that nerve fibers carrying vibration sensation are not affected in these patients.

(ii) Lepra bacilli, being “neurotrophic” and spread via the blood stream, may infect the periosteal nerves as well as the cutaneous nerves, giving a sense of painful paraesthesia to the periosteum. This possibility does not however explain why TS is usually negative in polar LL lesions overlying bone.

(iii) The periosteum of the involved area may be involved (periostitis) although the x-rays did not show any evidence of bone involvement. LL is known to cause an osteitis, but in TT, most of the bone changes described have been distal to a nerve trunk involvement or due to osteomyelitis (2, 3, 8). There was no clinical, radiological, or haematological indications of osteomyelitis in our patients. This possibility also does not explain why TS is not present in polar LL.

(iv) The affected cutaneous nerves, when pressed or crushed against the bone and an external hard object, may show a paraesthetic painful sensation. However, when the hypopigmented semi-anaesthetic lesions in tap sign positive areas were squeezed or lightly pinched with the index finger and the thumb, this sensation could not be elicited, indicating that the pathological explanation lies more in the bone.

TS is a user friendly test which can be used in the field clinics in developing countries. There is no added cost. A wooden paper weight or a similar object, or even clinician’s knuckles could be used. Comparison with the patients’ corresponding normal location makes assessment simple. This simple technique could be taught to healthcare workers who diagnose and treat leprosy in developing countries.

There are some limitations to TS as well. They include: BT and TT patients without lesions overlying bony prominences, extremely obese patients where bony prominences are difficult to find, and small children, debilitated, senile, or unreliable patients who can not respond to the questions about perceptions of pain.

In conclusion, “Tap Sign” is a useful clinical sign in the diagnosis of tuberculoid and
borderline tuberculoid leprosy where a lesion overlies a bone (sensitivity 66.7%). This test could be very useful to increase the clinical diagnostic yield, in the global perspective, in places where leprosy is diagnosed and treated by healthcare workers and primary care physicians without other laboratory facilities. Relative hyperaesthesia of the periosteum below the semianaesthetic lesions appears to be the cause. More research is needed to determine the exact pathophysiology of the pain sensation elicited by TS.

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In situ Hybridization in the Histological Diagnosis of Early and Clinically Suspect Leprosy

Mohan Natrajan, Kiran Katoch, Vishwa M. Katoch, Vishnu D. Sharma, Dharmendra Singh, RamDas, Devendra S. Chauhan, and Haribhan Singh

ABSTRACT

The present study tests the utility of the in situ hybridization procedure for M. leprae rRNA in the histological diagnosis of early leprosy and clinically suspect leprosy, both diagnostically demanding situations. The histological confirmation obtained with routine histopathology (Haematoxylin-Eosin staining for studying morphologic alterations and Fite-Faraco staining for demonstration of acid-fast bacilli) were 32% for early leprosy and 25% for clinically suspect leprosy. With performance of the in situ hybridization on the histologically unconfirmed cases, the positivity rates obtained were 58.8% and 55%, respectively. The results of the study confirm the utility of the procedure in the diagnostically difficult situations of early and suspect leprosy, and it is proposed that the procedure be employed in situations of clinical doubt.

RÉSUMÉ

Cette étude a eu pour but d’évaluer l’utilité de l’hybridation in situ des ARNr de M. leprae dans le diagnostic histologique de la lèpre précoce ainsi que de la lèpre cliniquement suspecte, des situations toutes deux très exigeantes en terme de diagnostic. La confirmation histopathologique obtenue par les techniques et lectures de routine (Hémalun-éosine pour étudier les lésions et le Fite-Faraco pour la mise en évidence des bacilles acido-résistants) a été de 32% pour la lèpre précoce et de 25% pour la lèpre cliniquement suspecte. Avec la mise en œuvre de l’hybridation in situ des cas non confirmés par l’examen histologique, les taux de positivité furent de 58,8% et de 55%, respectivement. Les résultats de cette étude confirment l’utilité de la procédure d’hybridation in situ dans les situations diagnostiques difficiles des lèpres précoces ou suspectes et il est proposé que cette procédure soit employée en cas de doute clinique.

RESUMEN

El presente estudio prueba la utilidad del procedimiento de hibridación in situ del RNA de M. leprae en el diagnóstico histológico de la lepra temprana y de la enfermedad bajo sospecha clínica, ambas situaciones difíciles de diagnosticar. La confirmación histológica obtenida por histopatología de rutina (hematoxilina-eosina para estudiar las alteraciones morfológicas y Fite-Faraco para la demostración de bacilos ácido-resistentes) fue del 32% para la lepra temprana y 25% para la lepra en sospecha clínica. En la hibridación in situ de los casos histológicamente no confirmados, las tasas de positividad obtenidas fueron del 58,8% y 55%, respectivamente. Los resultados del estudio confirman la utilidad del procedimiento en las situaciones de difícil diagnóstico de la lepra temprana y de la lepra bajo sospecha, y se propone que el procedimiento sea empleado en situaciones de incertidumbre clínica.
Leprosy is a chronic mycobacterial disease caused by *Mycobacterium leprae*. The diagnosis of the disease is primarily clinical and is dependent on the presence of two cardinal signs (8), namely, the presence of typical skin lesions with hypoaesthesia, and/or the presence of acid-fast bacilli (AFB). In the established form of the disease, the diagnosis is relatively simple, more particularly so when additional characteristic features such as typical skin lesions, cutaneous infiltration, and nerve thickening are present (23). In the early stages, however, the cardinal signs are yet to manifest and the diagnosis becomes problematic. There is thus the situation, more often encountered in areas of endemicity, where the diagnosis of leprosy cannot be made due to the absence of cardinal signs, yet the diagnosis of leprosy cannot be precluded as the lesion is too visually suggestive—a condition often referred to as “suspect leprosy” (24, 25). In such situations, the clinician resorts to histopathology to help resolve clinical doubt.

Routine histopathology requires for the confirmatory diagnosis of early leprosy, (i) the presence of infiltration within dermal nerves, or (ii) the presence of AFB, both of which are not uniformly present (27). Consequently, the histological confirmation of clinically early or suspect leprosy is possible only in a low percentage of cases. One way to augment the histological diagnosis is to use immunohistochemical (IHC) procedures which demonstrate the presence of mycobacterial antigens (20). As AFB are sparse in early forms and are rarely demonstrable in tissue sections despite serial sectioning, immunostaining, being a sensitive technique, can be used to demonstrate the presence of mycobacterial antigens in AFB negative specimens thereby pointing to the aetiology of the non-specific pathology seen (1, 21). There have, however, been some reservations expressed regarding the specificity of the procedure, particularly when anti–BCG is employed as the antigen-detecting antibody. *In situ* hybridization (ISH), first described by Pardue and Gall (11), is a powerful technique for demonstrating organism-specific nucleic acid sequences in tissue sections, and with the advent of non-radioactive and highly sensitive systems of labelling and detection (19), has become more amenable for diagnostic application on routinely processed paraffin sections (2). Nucleic acids specific to a variety of pathogens have been demonstrated in tissues using *in situ* hybridization (18). The present study tests the applicability and the usefulness of the procedure in the histological diagnosis of early and clinically suspect leprosy.

**MATERIALS AND METHODS**

Patients were selected from the those attending the out-patient department of the Institute. Only cases *not* having a history of prior consultation or treatment were chosen and these were clinically categorized using defined criteria (4, 23). The categories chosen were defined as: (i) indeterminate (Idt) leprosy, where the lesions were flat with vaguely defined margins and showing clearly demonstrable hypoaesthesia, (Hypoaesthesia was tested using the pin prick method.) (ii) Early Borderline Tuberculoid (BT) leprosy where the lesions were flat with margins defined in parts, and hypoaesthesia are clearly demonstrable. [(i) and (ii) constituted the clinical category of early leprosy.] (iii) Clinically “suspect” leprosy is leprosy in which the lesions were visually suggestive of leprosy showing the features in categories (i) and (ii), but without clearly demonstrable hypoaesthesia.

The clinical history and features were recorded, and slit-skin smears from the lesions were performed to examine for the presence of AFB (26). Incisional skin biopsies of the lesions were taken in 10% buffered formalin after taking written consent, and the tissue specimens were processed for routine histopathologic examination, i.e., for paraffin wax embedding, and subsequent Haematoxylin-Eosin staining for studying morphologic alterations, and Fite-Faraco staining for demonstrating AFB (28). Sections which, on such examination, showed a non-specific pathology (in the form of a mononuclear cell infiltration usually in the upper or mid-dermis, in perivascular and perianadnexal locations), and which were negative for AFB, were subjected to the *in situ* hybridization procedure. Glass slides coated with organosilane (APES, Sigma Cat. No. A3648) were used such that the tissue specimens would remain adherent to the slides during the harsh conditions necessitated during the use of the *in
*in situ* hybridization procedure. Apart from the test specimens, each set of slides stained contained a positive control in the form of a histologically AFB-positive specimen. The negative control consisted of the same AFB-positive specimen on which the same procedure was performed as with the other specimens but without the probe. A section of clinically normal skin from a healthy contact of a BT patient was additionally included.

The probes used were oligonucleotides, with the 18-mer (nucleotide length) oligonucleotide targeting the 16s rRNA of *M. leprae*, and the 20 mer targeting the 5s rRNA of *M. leprae*, and bearing the sequences: 5′ CTT CAA GGC GGA TGT CTT 3′ and, 5′ GGC TAC GGC GGG GGA CTC AA 3′, respectively.

The 16s rRNA targeting probe is recognized to be specific to *M. leprae* and has been used by several workers. The 5s RNA targeting probe was tested as an additional option.

The probes were procured commercially from a firm [Bioserve Biotechnologies (India)] to whom the desired sequence was provided. The oligonucleotides were synthesized using phosphoramidite chemistry and the product was verified by 12% polyacrylamide gel electrophoresis and optical density measurements. The oligos, which were supplied in a lyophilized state, were reconstituted in 600 μl of deionised water to give a concentration of 119 picomoles/μl in the case of the 16s RNA targeting probe, and a concentration of 112.8 picomoles/μl in case of the 5s RNA targeting probe. Oligonucleotides procured from the firm are currently being used in various studies at the Institute, either as primers for PCR studies targeting *M. leprae* and *M. tuberculosis* specific sequences, or as probes in membrane hybridizations (Southern and Dot Blot).

The probes were labeled with digoxigenin using the 3′ end-labelling method of oligolabelling, employing the enzyme terminal deoxynucleotide transferase (Roche Molecular Biochemicals, Cat. No. 1362372) which attaches a single molecule of digoxigenin to the 3′ end of the probe. Probe labeling was checked by direct detection on nylon membranes using the labeled control oligonucleotides provided in the kit as the standard (Fig. 1A). Probe specificity was checked by dot-blot hybridization using nylon membranes (Roche, Cat. No.1209299). Armadillo-derived *M. leprae* DNA was used as the target at a concentration of 0.25 ng/μl (vol. = 1 μl/spot) and the labeled oligoprobe at a final concentration of 1 ng/μl (vol. = 1 μl/spot) (Fig. 1B).

The *in situ* hybridization procedure was performed as detailed below. Section pretreatments: (i) sections were dewaxed, rehydrated and treated with 0.2N HCl to permeabilize the sections to the probe; (ii) proteolysis was performed with 500 μg/ml of pepsin in 0.2N HCl at 37°C for 20 min, the proteolysis stopped with 2% glycine, and the sections subsequently post-fixed with 4% paraformaldehyde for 20 min; (iii) acetylation with acetic anhydride in ethanalamine (0.2%) was done to quench free amino groups which helped reduce nonspecific signals; (iv) pre-hybridization was done using the hybridization mix in which the probe was not added, for a period of 2 hr at 42°C. The hybridization mix was prepared for a final volume of 3000 μl (3ml) for each set of slides with 50 μl being employed per tissue section.

The individual constituents of the hybridization mix and their final concentrations were as follows:

- **Denhardt’s Reagent**: 5× (1× Denhardt’s Reagent = 0.02% Ficoll + 0.02% BSA + 0.02% polyvinyl pyrrolidine)
- **SSC** 5× (SSC = Standard sodium citrate)
- **SDS** 0.5% (SDS = Sodium Dodecyl Sulphate)
- **EDTA** 1 mM (EDTA = Ethylene Diamine TetraAcetate)
- **Tris HCl (pH7.5)** 40 mM
- **Formamide** 25%
- **Carrier tRNA** 250 μg/ml
- **Dextran Sulphate** 10% (w/v)
- **PROBE** 1 μg/ml
- **De-ionized H2O** to make up vol.

Target denaturation was achieved by application of heat at 95°C for 3 to 4 min, which was followed by immediate, rapid cooling with ice.
Continuing the hybridization procedure:

(v) Hybridization was carried out at a final probe concentration of 1 µg/ml of hybridization mix, at 42°C, applied overnight (15 hr).

(vi) Post hybridization washes were done with Standard Sodium Citrate (SSC, of strengths 2×, 1× and 0.5×) containing 0.1% sodium dodecyl sulphate (SDS), over a time period of 45 min.

(vii) Visualization was done using the enzyme alkaline-phosphatase and with NBT/BCIP (Nitro-Blue-Tetrazolium/Bromo-Chloro-Indoyl-Phosphate) as the substrate chromogen.

(viii) Two percent Neutral Red was used as the counterstain, and, DPX, a synthetic resin, was the mounting medium employed.

RESULTS

Twenty-five cases each from the clinical categories of early leprosy (Idt = 15, BT = 10) and suspect leprosy, were included in the study. The cases were all adults (age ranges 15 to 50 yrs for both categories), predominantly male, and all negative for AFB on skin smear examination. Routine histopathology confirmed the diagnosis of leprosy (all histologically Indeterminate) in 8 (32%) of the cases of early leprosy with AFB being seen in 2 cases; in clinically suspect lesions, histological confirmation was possible in 5 (20%) cases with AFB being seen in a solitary case. In situ hybridization performed in the remaining cases with the 16s rRNA-targetting probe yielded positive signals in 10/17 (58.8%) cases from the early leprosy category and in 11/20 (55%) of the clinically suspect cases (The Table). The positivity rates obtained with the 5s rRNA-targeting probe were closely similar with positive signals being seen in 9/17 (52.9%) cases of early leprosy and in 12/20 (60%) cases of suspect leprosy.

Positive signals, viewed as deep blue precipitates of the NBT/BCIP-Alkaline Phosphatase reaction, were easily discernible against the red background of the Neutral Red counterstain, and were found to be mostly located among the cells of the dermal infiltrates (Figs. 3, 4, 5). The morphology of the tissue specimens was fairly well preserved and the signals could be located in the context of tissue pathology. Background and non-specific staining were minimal, and the latter was usually seen in the cells of the basal epidermis, eccrine glands,
and occasionally in the nuclei of the inflammatory cells. Dark-blue thick NBT/BCIP crystal precipitates were at times seen, but could be easily distinguished from the specific signals by virtue of their morphology and bizarre locations. Any ambiguous staining seen was treated as a negative result.

The specificity of the signals was supported by the control results—positive results in the positive control, and absence of signals in the negative control and in normal skin. The specificity of the probes had already been confirmed by membrane blotting as described earlier.

**DISCUSSION**

There has been a decline in the number of leprosy cases worldwide (36), and concomitant with it there has been a rise in the fraction of both early cases and of clinically suspect cases. Both are situations where diagnostic difficulties are encountered. Routine histopathology’s help in these situations is rather limited, a fact being borne out by several studies (10, 24, 34). In a well documented epidemiological study conducted in Malawi, the histological confirmation of suspected cases of leprosy varied from 29% to 58%, with the percentage confirmation being strongly dependent on the degree of clinical suspicion involved. The clinical categories in the present study were chosen by clinicians using defined criteria, and clinical suspicion was accorded by more than one clinician, which helped maintain a degree of homogeneity in the series of cases studied. The observed histopathological confirmation rates in the present study are slightly lower, which is not unusual given a different environmental situation.

As stated earlier, immunohistochemical staining procedures which demonstrate mycobacterial antigens can be used to augment the diagnosis, particularly in situations where AFB are not demonstrable (1, 14, 21). The contribution made by the use of these procedures is noteworthy but limited, with immunostaining positivity in AFB negative specimens averaging about 35% in the studies quoted above. This leaves a significant number of cases diagnostically unconfirmed.

**In situ** hybridization offers a very high specificity and a high sensitivity, and, as stated earlier, has been put to widespread use in the diagnosis of bacterial and viral infections; in some situations, it is clearly more sensitive than immunohistochemistry (30). The procedure has, however, not been tested on tissue sections for its diagnostic utility in leprosy and there is a general perception that the procedure is not sensitive enough to contribute to the diagnosis of leprosy, particularly the paucibacillary forms.

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**THE TABLE.**  *Data resulting from routine histopathology and in situ hybridization study of early and clinically suspect leprosy.*

<table>
<thead>
<tr>
<th>16s rRNA targeting probe</th>
<th>Clinical category</th>
<th>No. of cases</th>
<th>Diagnosis of leprosy (routine HP)</th>
<th>% HP positive</th>
<th>Nos. ISH tested</th>
<th>Nos. ISH positive</th>
<th>% ISH positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early leprosy (Idt, BT)</td>
<td>25</td>
<td>8</td>
<td>32</td>
<td>17</td>
<td>10</td>
<td>58.83</td>
<td></td>
</tr>
<tr>
<td>Clinically suspect leprosy</td>
<td>25</td>
<td>5</td>
<td>20</td>
<td>20</td>
<td>11</td>
<td>55</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5s rRNA targeting probe</th>
<th>Clinical category</th>
<th>No. of cases</th>
<th>Diagnosis of leprosy (routine HP)</th>
<th>% HP positive</th>
<th>Nos. ISH tested</th>
<th>Nos. ISH positive</th>
<th>% ISH positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early leprosy (Idt, BT)</td>
<td>25</td>
<td>8</td>
<td>32</td>
<td>17</td>
<td>9</td>
<td>52.9</td>
<td></td>
</tr>
<tr>
<td>Clinically suspect leprosy</td>
<td>25</td>
<td>5</td>
<td>20</td>
<td>20</td>
<td>12</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>
In the present study however, positive signals could be obtained in 58.8% of cases of early leprosy and in 55% of cases of clinically suspect leprosy where the histological features were nonspecific. Viewed in the context of expectations, the positivity rates would appear high. Though the relatively high positivity rate could be attributable to
a certain extent to a hospital-based bias in the selection of cases, high positivity rates with the *in situ* hybridization procedure are not unknown. Positivity rates of the order seen in the present study have been reported in varied situations. Investigators have reported the detection of *M. avium* subspecies *paratuberculosis* in 40% of diseased tissues

![Image](image1.png)

**FIG. 4.** Positive hybridization signals seen in the upper derma infiltrate of a tissue specimen from a case clinically diagnosed as indeterminate leprosy where routine histopathology was nonspecific and AFB absent. (Detection system—Alkaline phosphatase—NBT/BCIP. Counterstain: Neutral Red. ×670).

![Image](image2.png)

**FIG. 5.** Positive hybridization signals are seen in the subperineurial region of a dermal nerve in a case of clinically suspect leprosy. Routine histopathological examination revealed a non-specific pathology and AFB were absent. (Detection system—Alkaline phosphatase—NBT/BCIP. Counterstain: Neutral Red. ×468).
from patients with Crohn's disease using in situ hybridization (15); a more recent study reported a positivity of 73% in similar cases and in whom, additionally, granulomas were absent (32). Cytomegalovirus DNA could be seen in 9 of 13 colonoscopic biopsies which were histologically negative (29), and Human Papilloma Virus was demonstrable in 8/11 histologically normal tissue of patients with genital cancer (17). Though exact parallels cannot be drawn from these studies, it cannot be denied that the in situ hybridization procedure is sensitive enough to give good positivity rates and that it works in histologically negative situations.

Oligonucleotides were chosen as the probes because they penetrate well into tissues. The particular oligonucleotides used in the study were chosen from a panel of M. leprae targeting probes bearing sequences complementary to those encoding parts of the 18kDa protein (35), 36kDa protein (13), 16s rRNA (3, 16), and 5s rRNA of M. leprae, and whose specificity was confirmed using dot-blot hybridization. Each of these oligonucleotides were developed for use as primers in procedures employing the polymerase chain reaction (PCR), which held great promise in the diagnosis of paucibacillary states where the causative pathogen was not demonstrable. Several studies have been performed and replicated on a variety of specimens ranging from mouse footpad biopsies, to biopsies of skin lesions from patients, either in the form of frozen sections or paraffin-embedded sections. Importantly, the procedure could be performed on ethanol-fixed and neutral-buffered formalin fixed tissue specimens. Detection limits have varied between 1 and 100 bacilli, and the positivity rates in biopsy specimens from paucibacillary leprosy with a BI of 0 have varied between 35% and 73%. Thus, from a purely diagnostic point of view, PCR on material extracted from tissues would be simpler and more sensitive compared to the in situ procedures. The constraints lie in the distinct possibility of false positive results due to external contamination and in the lack of structural correlation, both of which are obviated with use of in situ procedures.

The probes targeting 16s rRNA and 5s rRNA were found to perform the best, producing strong, consistent signals during standardization, and were therefore used in the present study. The 16s rRNA of M. leprae is known to bear sequences specific to

**FIG. 6.** Positive hybridization signals seen in the perineural infiltrate of a skin biopsy specimen in a case clinically diagnosed as early leprosy; acid fast bacilli were not demonstrable. (Detection system—Alkaline phosphatase—NBT/BCIP. Counterstain: Neutral Red. ×670).
the organism, which has been exploited as an identification tool in several studies (16, 22, 33); the 5′ rRNA-targeting probe which performed equally well provides an additional option for use.

Digoxigenin was chosen as the label because of its high sensitivity, which approaches that of radioactive labels (21). The 3′-end labelling and tailing methods of oligonucleotide labeling were tried and both the methods were found to perform equally well despite the latter’s avowed higher sensitivity; the 3′-end labeling procedure was eventually employed. The detection system employed—the alkaline-phosphatase NBT/BCIP system—is recognized to be the most sensitive (6), and brief dips in alcohol and xylene, which are necessary subsequent steps, did not wash away the blue-black end product. The color of the end product precluded the use of Haematoxylin (the ideal) as the counterstain, and 2% Neutral Red was used in its stead.

In addition to the categories of specimens chosen for the study, the in situ hybridization procedure was performed on histologically confirmed cases to get an idea of the overall sensitivity of the procedure. Of the ten cases studied, 2 cases were negative by ISH, but in both the cases the histologic diagnosis was based on morphological alterations, and not on the presence of AFB. The sensitivity of the procedure is therefore high and the specificity was indicated by the absence of signals in the negative controls as well as in normal skin.

Some aspects of the in situ hybridization procedure that were brought to the fore during the performance of the study deserve mention. A major consideration is the technical difficulty wrought by the length of the entire procedure, which extends into the third day. Occasionally, the procedure itself may fail, or the tissue may be either lost or suffer gross disruptions in its morphology. These are related to the necessarily miniscule volumes of the reagents employed during the entire procedure, and to the relatively harsh conditions which prevail during the performance of target denaturation, and during the pre-hybridization and hybridization steps of the procedure. Nevertheless, these encumbrances should not be a deterrent to the effective use of the procedure, as the yields more than compensate for the efforts expended. The procedure is well worth considering for use in situations of clinical doubt.

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Nineteenth Century Indian Leper Censuses and the Doctors

Shubhada Pandya

ABSTRACT

This study describes the circumstances under which enumerations of “lepers” were conducted in India in the late 19th century, and the ideological biases of the respective investigators and the meanings that they read into the statistics. This report focuses on the Bombay Presidency leprosy returns of 1867, examined in 1871 by Henry Vandyke Carter, and the decennial nation-wide population census of 1871–1872, 1881, and 1891, in which the leprosy-affected, among other infirm persons, were also enumerated. The evidence examined includes the investigators’ reports and other published and unpublished contemporaneous documents. These censuses were undertaken at a time when the etiology of leprosy was a major controversy, but the evidence here indicates that the efforts to clarify the etiology and estimate the virulence of the disease in India by means of statistics were animated by the desire to justify and embellish pre-conceptions. Despite the claim that they were necessary for leprosy control, the censuses, for various reasons, were not utilized towards that end in India.

RÉSUMÉ

Cette étude décrit les circonstances selon lesquelles le dénombrement des « lépreux » furent menées en Inde à la fin du dix-neuvième siècle, les biais idéologiques des investigateurs respectifs et les interprétation qu’ils tirèrent des statistiques. Ce travail s’est focalisé sur le rapport officiel de lèpre de 1867 sous la Présidence de Bombay, examiné en 1871 par Henry Vandyke Carter, et les recensements nationaux de 1871–1872, de 1881 et de 1891, au sein desquels les personnes souffrant de lèpres, parmi d’autres populations infirmes, furent dénombrées. Les faits examinés ont inclus les rapports des investigateurs ainsi que les autres documents contemporains publiés ou non. Ces recensements ont été entrepris à une époque où l’étiologie de la lèpre était encore hautement controversée; cependant les données examinées ici indiquent que les efforts pour clarifier la cause et estimer la virulence de la maladie en Inde au moyen de statistiques, étaient motivés par le désir de justifier et embellir des idées reçues. En dépit de l’assertion que les recensements étaient nécessaires pour pouvoir mieux contrôler la lèpre, ils ne furent pas, pour de nombreuses raisons, utilisés dans ce but en Inde.

RESUMEN

Este estudio describe las circunstancias en las cuales se contabilizaron los pacientes con lepra en la India a finales del siglo 19, los factores ideológicos de las respectivas investigaciones y el impacto que estos datos tuvieron en las estadísticas de la enfermedad. El reporte se enfoca a los registros de la lepra en 1867 que fueron examinados en 1871 por Henry Vandyke Carter, y en los censos deceniales de la población nacional de 1871–1872, 1881, y 1891, en los cuales se enumeraron las personas afectadas de lepra, y otros enfermos. Las evidencias examinadas incluyen los reportes de los investigadores y otros documentos contemporáneos publicados y no publicados. Estos censos se hicieron en un tiempo en que la etiología de la lepra era controversial y las evidencias aquí presentadas indican que los manejos estadísticos para clarificar la etiología y estimar la virulencia de la enfermedad en la India estuvieron animados por el deseo de justificar y embellecer ideas preconcebidas. No obstante la justificación de que eran necesarios para el control de la lepra, los censos, por varias razones, no fueron utilizados con ese fin en la India.

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[Note: The use of “leper” in this paper is solely on account of the historical context; there is no intention to disparage the person with leprosy].
An interesting aspect of the leprosy censuses conducted in nineteenth century colonial India is the medical connection. The present paper examines this subject, describing the circumstances under which the enumerations were conducted and focussing on two instances when census statistics were scrutinized and variously utilized by British physicians of the period. The first instance relates to the Bombay Presidency leprosy returns of 1867, which were examined in 1871 by Henry Vandyke Carter of the Bombay Medical Service. The second instance deals with the decennial nation-wide (“Imperial”) population censuses of 1871/72, 1881, and 1891, in which the leprosy-affected, among other infirm persons, were also enumerated. The general censuses deeply interested the Leprosy Commission which visited India from 1890 to 1891. An important objective of the present paper is to demonstrate the close correlation between the ideological biases of the respective investigators and the meanings that they read in the statistics. The information sources are the investigators’ reports and other unpublished and published nineteenth century material. Background reading of two recent histories of leprosy in the Indian colonial context (by Jane Buckingham and Sanjiv Kakar, respectively) suggested that the proposed approach to the subject has not been considered before (9, 18). Buckingham, while devoting considerable attention to Carter, does not cite his census analysis of 1871 (referred to above). Kakar, like Buckingham, elaborates on the medico-political milieu in which the visit of the Leprosy Commission took place, and recounts the chief features and long-term consequences of its conclusions and recommendations, but does not deal with its members’ ideological biases.

The two examinations of census statistics were undertaken at a time when the etiology of leprosy was a major bone of contention between investigators in Europe, the dominant ideologies in the debates being hereditarianism, sanitarianism and contagionism (22). Hereditarianism gained respectability as a result of the pioneering 1847 treatise of the Norwegians Danielssen and Boeck who were impressed by the high frequency of familial leprosy in their study subjects (15). The European debates and disagreements echoed in India. It has been held that the periodic classification and enumeration of the indigenous population was a strategy by which Britain’s “Empire of Information” in India sought to “know,” hence better control, its subjects (8, 13). “Knowledge is power” was a phrase familiar to Victorian Era imperialists. In England in the 1860s in the medical discourse on leprosy in the colonies, “knowledge” about leprosy numbers was equated with “humanity” and as an imperial duty towards the subject population (5). In 1856, Charles Morehead, principal of the Grant Medical College, Bombay, in his much praised Clinical Researches on Disease in India had to be content with opening the section on leprosy with the general statement that, “leprosy is common enough in India” (21). By the end of the century, the situation had changed; authors were able to endow their works with some precision by citing the results of several Indian leper censuses (16). Leprosy statistics were seen as a guide to the “truth” about Indian leprosy.

The Bombay Presidency “Leper” Census of 1867 and H. V. Carter. The need for precise information on leprosy in the Presidency was voiced in early 1866 by A. K. Nairne, Assistant Collector of the coastal district of Ratnagiri, pointing out to his superior officer the pitiful condition of the numerous lepers in the streets of the main town. From an informal census conducted by him in fifteen of the larger towns and villages in the district, Nairne said he estimated that there were at least 200 sufferers, 50% of them in such an advanced stage, that he could “scarcely describe the loathsomeness of the unfortunate creatures” (3). As a result of the suggestion, a more extensive census was launched by the Presidency Sanitary Commissioner in 1867. The itemized information sent in by village police from almost all districts of the British governed regions of the Presidency, related to residence, sex, age, and caste of each “leper.” In deference to the hereditary theory of causation, the returns contained a special entry on whether or not there was a similarly afflicted relative (blood, conjugal, or other) in each case. The object of the exercise, it was said, was to “collect definite data upon which to base measures, if these could be
carried out, for the relief of the leprous, and possibly in due course, the protection of the community against the spread of the disease” (3). Information on 8,220 lepers became available as a result, but lay unutilized in government files for some years until Carter (1831–1897) (Fig. 1) volunteered to collate and analyze it. At the time, he was chief medical officer (“civil surgeon”) in the district of Satara on the Deccan plateau. Unlike his peers in the Service, Carter was not attracted to private practice, hence his remark that he had time enough on his hands to undertake the task. Published in 1871, his report took up over 170 pages in the Transactions of the Medical and Physical Society of Bombay (12). The analysis was undertaken during the peak of his confidence in the hereditary theory of causation, in which of course he was not alone.

Although it could not pretend to have perfect accuracy, Carter thought that the data was reliable enough to warrant “laborious scrutiny.” He found it noteworthy that, although the disease was present throughout the Presidency and affected all communities, the prevalence rates varied widely, not only between regions but within sub-regions.
as well. It averaged 1 per 1000, ranging from 1 per 2500 to 1 per 428, the so-called “Maratha country” in the south Konkan and the adjoining Deccan regions showing the highest intensity, and Sind in the north the lowest. Within districts too, disease foci were found in hamlets with but a few hundred inhabitants (12) (Fig. 2). Examining the bearing on aetiology, Carter swiftly discounted external factors such as local geography, topography, or climate as relevant. Inter-current diseases such as malaria, cholera, and syphilis also appeared not to be implicated. Neither was poverty, and Carter took pains to point out that want and deprivation were consequences rather than precedents of leprosy. Significantly, he saw no lepra-genic potential in the “habits” of the people, which were “very regular;” Indians bathed once a day; and “personal cleanliness is not neglected” (12). Such statements were a direct refutation of sanitarianism. Carter passed over the possibility of “contagion” as an alternative explanation for the presence of disease foci, with the brief comment that it required “further study.” He gave a similarly succinct response to the hypothesis offered earlier by a Calcutta physician that

Fig. 2. Carter’s Leprosy Map of Bombay Presidency showing regions of high prevalence (Original is in color).
leprosy was contagious through the “casual inoculation of leprous matter” (20). That possibility, Carter declared, was un-provable except by an impossible experiment (12).

Carter’s rejection of sanitarian views in etiology did not amount to complete abolition for the “natives.” He was convinced that “the cause [of leprosy] must be searched for in the people themselves” (12). The pathologizing of the Indian body followed the logic of the hereditary doctrine to which he had unreservedly subscribed from as early as 1863: “Leprosy in all its varieties is decidedly hereditary” (10, 11). The entries in the returns relating to the presence or absence of leper relatives were thus of the greatest importance, and several pages were devoted to cross-tabulations of “heritage” against the other variables. Momentarily disconcerted that only about one-fifth of the lepers (1564 out of 8220) had acknowledged a “hereditary taint” in a blood relation (most often in the male direct line), Carter hastened to claim that this was an underestimate, his conviction being that the “role of hereditary [influences] . . . must be supreme” (11). He likened the putative hereditary leprous “taint” to a “force of many degrees” with a single direction, namely from parent to child (11). His speculations were undoubtedly aided by the fact that no definition existed about what constituted a “hereditary disease,” the term being applicable to any disease which was present in more than one generation of a family (17). Sheltering under the vast umbrella of “hereditary transmission” was unproblematic for Carter; “it is only when any peculiarities . . . are in appearance not derived by inheritance, that an explanation of their origin is really difficult,” he proclaimed (italics in original) (12). Manifold influences were known to act on inheritance. Carter conceptualized the “leprous taint” as subjected over generations to endless modifications by external adverse or favorable influences, opposed or reinforced parental qualities, which deferred or accelerated the development of the disease in the offspring (12). He claimed that the phenomenon of atavism (reversion to the characters of an ancestor), explained why a grandparent or great-grandparent alone was sometimes named by a leper as having been afflicted. The hereditary influences on the “taint” being numberless, the non-inheritance of leprosy, though it was much more frequently asserted than the converse, [as] . . . likely to be apparent rather than real, that this disease commonly arises spontaneously—i.e., independently of parental influence—[is an] . . . assumption fitted more to confuse than to make clear (12).

So-called “spontaneous” disease (familial leprosy denied, comprising 80% of those enumerated), was, according to Carter, an instance of atavism. He clinched his case by arguing that the “spontaneous” and “transmitted” forms of leprosy resembled each other so well as to be identical; since one was transmitted by inheritance, “it must necessarily follow that there is a similar originating cause for the other” (12). The “lepers” who denied a family history were simply not telling the truth—an understandable lapse, in view of “a natural objection to acknowledge the presence of a loathsome family taint” (12).

The most notable feature of Carter’s analysis was not his “proof” of hereditary transmission, but his stepping out beyond biology into ethno-sociology. He discovered the fount of Indian leprosy in a subject “full of instruction to the antiquary and the ethnologist,” namely the country’s caste system. Caste, the Hindu system of social stratification, was a mine little worked in the cause of medicine, but which promised to be a resource for a detailed analysis of many curious anomalies in the “opinions and conditions of the native races,” he maintained (12). He made much of the fact that caste distinctions, foreign and artificial, though they appeared to Westerners, were tenaciously adhered to by Indians, even by those lowest in the hierarchy. He recounted the racial ancestry of the indigenous people on the basis of conventional Orientalist wisdom, which claimed the bulk of Hindus in Western India were descendants of Caucasian conquerors, with a “leavening” by Aryans, who had spread over the country after subjugating the aboriginal tribes. Some descendants of Oceanic and Mongol stock, which entered the country earlier through the Eastern Himalayas, were represented in Western India by the higher caste Marathas, and a large complement of lower castes like the Mahars with those in a “ruder state” like the aboriginal Warlis and Katkaris of Thane district. Prevalence rates of leprosy were high
in these groups, Carter reminded readers. But since the descendants of the Caucasian invaders were also not free from leprosy, Carter declared that the disease reached India in various ways (such as the Mahomedan invasions), and at various historical times resulting in a “tainting” of the whole population and pockets of high prevalence. Carter blamed caste mores for rendering the disease endemic with no tendency to subside. The customs he seized on as the vehicle for hereditary transmission were endogamy and exclusivity, the supposed linchpins of the caste system. There were some castes, he alleged, in which marriages were preferentially contracted between families who had intermarried for several previous generations. Incest was abhorred; yet in circumstances when the prescribed lineage distance could not be adhered to, closer intermarriages were practiced. The marriage rules of the “ruder” groups such as Mahars and tribal people of the Deccan and Konkan were still more lax, accounting for their proneness to the affliction.

It was accepted practice and expedient for the colonial British to view Indians through the prism of caste, lacking individuality or agency. Carter sought to invest his theorizations with added weight by recruiting John Wilson, a prominent Scottish missionary and educationist in Bombay, and the future author of a work titled *Indian Castes*, to revise and annotate the list of “Leper Castes” in the “Report” (12). The Indian “leper” thereby became comprehensible to Westerners by his fixed place in the caste map. In the Carterian scenario the Indian body, burdened with the leprous seed from prehistory, shackled by caste, endogamous to the point of incest, could never free itself of the taint; Indian leprosy was a paradigm of ethno-sociological determinism.

The “Imperial” Decennial Censuses and the Leprosy Commission in India (1890 to 1891). The enumeration of infirm persons such as the blind, deaf-mutes and insane as part of the decennial population censuses undertaken from 1871 to 1872 in colonial India, was modelled on the practice in Europe (including Britain) and the Americas (7). The inclusion of lepers among “infirm” persons was unprecedented, a probable result of the ubiquity of the disease and colonial officials’ expectation that leprosy was well recognized even by unprofessional Indian enumerators and the unlettered public. But as early as the 1860s, W.W. Hende, Civil Surgeon at Nagpur in Central India had warned logical-minded British statisticians that leper enumeration in India was fraught with imprecision:

This not an imaginary idea . . . when it became known that the inquiry was to be instituted, nearly 200 lepers at once left the city, in consequence of a malicious report having been spread that . . . the Government wished to catch all “leper’s and ship them off . . . (Report on Leprosy of the Royal College of Physicians, p. 86).

And indeed, every census report in India admitted to serious errors of omission and commission in the enumeration of lepers. In 1871 and 1872 in Bombay, for example, bureaucrats bungled their instructions to the enumerators, with the result that persons with “white” (leucoderma) as well as “true leprosy” were entered simply as “lepers.” In 1882, Jervaise Baines, the Presidency Census Commissioner, cautioned against unquestioning acceptance of the data because, of “all the particulars returned at the enumeration, there are none . . . even the ages . . . so incomplete and vague as those regarding infirmities” (7). Besides continuing to confuse leucoderma and “true leprosy,” Baines complained that the enumerators were unable even to recognize “true” leprosy until it was at an advanced stage. Another source of error, underreporting, was found to be “lepers’” families fears that the enumerator would abuse the information. Statistics about female lepers were suspected to be particularly suspect, attributable to a special reluctance on the part of families to divulge the correct information, with the result that only about 30 women lepers were recorded for every 100 male, quite disproportionate to the sex ratio in the general population (7). Reporting on the 1891 leper census operations Baines, (by then promoted as Imperial Census Commissioner), attempted answers to two questions, namely “How many lepers?” and “Is their number increasing?” concluding that it was:

impossible to say for certain whether this disease is, in the whole, stationary or not . . . It does not seem advisable to enter into further analysis here in the case.
of a disease of which little is known and regarding which the returns are possibly inaccurate (6).

Father Damien died in 1889 of leprosy contracted in Hawaii. At one stroke, Indian leprosy acquired a fearful relevance and immediacy for lay and medical ultra-contagionists and alarmists in Britain. Archdeacon Henry Wright warned that the rampage of Indian leprosy went unheeded by England at her own peril. In a booklet emotively titled, Leprosy an Imperial Danger, Wright sketched a doomsday scenario:

The Indian lascar... finds his way to the seaport or larger city slum... and the boarding house dens with their insanitary promiscuity. The process once fairly set going and the necessary time allowed, should we be surprised to find leprous centers of contagion forming themselves once more within our limits?... One more loud and warning cry, England! Beware! (26).

Other panic-mongers claimed that the “real” number of lepers in India was far higher than suggested by official figures, and was growing. The Cassandras were in august company. The medical, political, and ecclesiastical elites of England meeting under royal patronage to set up the Father Damien Memorial National Leprosy Fund in 1889 were reported to have heard with a “thrill of horror and incredulity,” the Prince of Wales’ disclosure in the Times of India that a “leper” was employed in the London meat market (8 July 1889). No less than Queen Victoria’s physician was to declare in the Times of India that not only had leprosy increased in recent years, but new “centers” were springing up, that before England and the “civilized world” there loomed a great threat (5 February 1890).

In late 1890, a three-member Commission (aided by two officers of the Indian Medical Service who joined them in India) was despatched to India by the National Leprosy Fund, with a mandate to investigate the disease including its cause, and to recommend measures for its control and containment (1). The composition of the Commission was significant; (i) Alfredo Antunes Kanthack (1863–1898) (Fig. 3), laboratory scientist, was the nominee of the Royal College of Surgeons of England. After qualifying in 1888, he proceeded to the F.R.C.S. a year later; prior to his appointment on the Commission he had spent a year acquiring research experience in Berlin under the pathologist Rudolf Virchow and the bacteriologist Robert Koch. (ii) George Alfred Buckmaster (1859–1937) (Fig. 4) was nominated by the Executive Committee of the National Leprosy Fund. He obtained an M.D. from Oxford in 1887, and worked in physiology laboratories in Germany. He was at the Department of Physiology and Anatomy at St. George’s Hospital in London when called to join the Commission. (iii) Beaven Neave Rake (1858–1894) (No photograph available), the most influential member of the team was the nominee of London’s Royal College of Physicians. He earned M.D. from London University in 1882, and thereafter spent time in Vienna and Berlin. In 1887, he joined the Colonial Medical Service as Medical Officer in charge of the Leper Hospital in Trinidad. Rake had written frequently on the disease including its spread, the bacillus, and human and animal inoculation experiments, and was thus the only Commissioner who could claim practical experience in leprosy. The Royal College of Physicians certainly had a vested interest in Rake’s nomination. That august institution was the bastion of opposition to the contagionist doctrine, its credentials in this regard having been established in 1867 in its famous Report on Leprosy, and re-iterated in 1874 and 1887 (4, 14, 15). Rake had expressed scepticism about the contagious (i.e., by contact) spread of leprosy. As early as 1875, the “British Guiana Leprosy Commission” of thirteen members agreed unanimously that leprosy was contagious. Rake said he was unconvinced. A crucial human inoculation experiment was conducted in November, 1885, when George Arning a German physician working in Hawaii inoculated a convicted murderer, Keanu, apparently in good health, with leprosy bacilli. In due course, it was reported that leprosy had developed in him (25). Much importance was attached to this development by contagionists as it seemed that the disease was accounted for by the inoculation. It became subsequently known that Keanu had several leprosy-affected relatives. With contagionists in full cry it required strong convictions
to question majority opinion on the Damien and Keanu cases. The battle was joined. Rake maintained that both cases having arisen in a leprosy-endemic area (Hawaii), were of no value as evidence of disease transfer by contact. He argued that the infection might have entered Damien’s body by other routes:

The well-known case of Father Damien had been very generally accepted as tending to prove contagion. To my mind, this is very far from conclusive . . . He may . . . have absorbed the specific virus (generally believed to be the _bacillus lepræ_) in many other ways, e.g., in food, water, air, etc. (23).

The Leprosy Commissioners toured the entire country from November 1890 to March, 1891, examining and questioning almost two thousand “leprosy sufferers.” On completing their investigations in early 1891, they deliberately withheld their final
report to await the result of the census of 1891. Their anti-contagionist sympathies made them acutely aware of the importance of countering alarmist outcries on the “Imperial danger” issue, and the case would be decided on the basis of census figures. In order to disarm potential critics they acknowledged at the outset in their Report that the trustworthiness of the statistics might be questioned, but insisted that there was merit in the figures. In the first two censuses, (of 1871/72 and 1881) “the diagnosis was greatly at fault,” but with time “the diagnostic powers of the people [to distinguish between true leprosy and leucoderma] improved,” and such errors were minimal in the census of 1891 (1). The figures were valuable, possessing, if not an absolute, at least a relative value, which was greatly enhanced in succeeding censuses. For example, certain regions of the country had consistently returned the highest prevalence rates in each census. The strategy adopted was to deal in prevalence rates rather than actual “leper” numbers. In the interest of uniformity they confined their

Fig. 4. George Alfred Buckmaster (1859–1937) member of the Indian Leprosy Commission (1890–1891).
calculations to the districts in “British India,” which had been enumerated in all three decennial censuses (The Table). However, an anomaly is immediately noticeable in the figures presented by them: in the 1891 census the total population of the enumerated districts exceeds the total population of “British India.” This demonstrates not only the unreliable statistics but the anti-contagionist Commissioners’ eagerness to utilize them in order to “prove” that:

The evidence of the censuses excludes the idea of an increase of leprosy, and points rather to a gradual decrease at the present time . . . Anyway an “Imperial Danger” leprosy has not become as yet, for the previous increase is more than counterbalanced by the present decrease (1).

There is good reason to see Rake’s hand in the pointed contesting of Archdeacon Wright’s “Imperial Danger.” Some months earlier in the “Annual Report of the Trinidad ‘leper’ Asylum,” the leprologist had pointed out another India-related exaggeration in the cleric’s work, namely that people of Indian origin in Trinidad were “readily attacked” by leprosy (24). Discounting alarms that the un-confined and un-segregated Indian “leper” was a threat to Britain and its imperial interests in India was consistent with Rake’s scepticism regarding the special vulnerability of Indians to the disease. The down-playing of the “Imperial danger” also provided a foretaste of the tenor of the Commission’s final conclusion on contagion (contact) in leprosy transmission.

**CONCLUSION**

This paper has highlighted the crucial role that the ideological biases of the respective investigators played in their studies of the censuses. The efforts to clarify the etiology and estimate the virulence of the disease in India by means of statistics were animated by the desire to justify and embellish preconceptions. The two analyses were conducted two decades apart in differing prevailing climates of opinion regarding etiology and mode of transmission. In a demonstration of medical imperialism in 1862 the *British Medical Journal* opined that the Orient was infested by leprosy, “to a greater or less extent, . . . in proportion to the physical and moral degradation of [the] people” (6 December 1862). The hereditarian Carter’s formulation of Indian leprosy as being grounded firmly in Indian ethno-sociology was at one with the Journal’s allegation. The visit of the Leprosy Commission on the other hand took place in the post-Damien period when hereditarianism had lost ground and imperialist sentiment was framed in contagionist terms. It is notable that the long-discovered leprosy bacillus had hardly any place in ultra-contagionist polemics of 1889 and 1890, and a European priest’s leprosy provided the fuel and the fodder. The Leprosy Commission’s scepticism about contagion (contact), its anxiety to refute at the outset of its Report, allegations of “imperial danger,” though counter

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**THE TABLE. Compiled from Table I (p. 48) and Table I-b (p. 56) in the Commission’s Report. In 1891, the population of the districts in which leprosy sufferers were enumerated exceeds that of “British India.”**

<table>
<thead>
<tr>
<th>Total population at each census on which the leper ratios have been calculated</th>
<th>No. of Lepers</th>
<th>Ratio per 10,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Census (1867–1872): 182,000,000 (population of “British India”: 184,056,627)</td>
<td>100,000</td>
<td>5.0</td>
</tr>
<tr>
<td>2nd Census (1881): 101,000,000 (population of “British India”: 196,895,542)</td>
<td>15,000</td>
<td>6.0</td>
</tr>
<tr>
<td>3rd Census (1891): 210,000,000 (population of “British India”: 208,242,300)</td>
<td>105,000</td>
<td>5.0</td>
</tr>
</tbody>
</table>

“Remarks: Only those districts and areas have been included which were enumerated in all censuses. To facilitate calculation round numbers have been used, and the ratios are approximate only. It has been assumed that the number of lepers in the Central Provinces has remained stationary.”
to the dominant wisdom, was predicated on the avowed anti-contagionism of its influential member.

Turning to the censuses themselves, one might ask why the colonial authorities, knowing full well that the enumerations were unreliable even on basic information, persisted in their self appointed task of counting “lepers,” decade after decade. (Indeed the practice was discontinued only in 1931). Possibly it was the bureaucratic momentum that propelled the enterprise through seven all-India exercises, possibly it was the belief that faulty statistics were better than no statistics at all. Despite the claim that they were necessary for leprosy control, the censuses, for various reasons (the Leprosy Commission’s views among them), were never utilized towards that end in India.

Acknowledgment. This is an expanded version of a paper read at the Annual Conference of the European Association of the History of Medicine and Health held in Oslo September 2003. I thank the Global Project on the History of Leprosy for making it possible for me to attend.

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This issue of THE JOURNAL carries an important contribution by Cairns Smith and colleagues, describing a large and unique epidemiological study carried out in three leprosy endemic villages on Maharashtra State in India. The study addresses the role of the upper respiratory tract in the transmission of Mycobacterium leprae, using two assays: PCR for M. leprae antigen detection on nasal swabs and salivary IgA for mucosal immune response to a crude whole M. leprae antigen. The results are interpreted as consistent with an hypothesis that M. leprae persists in endemic communities as transient, benign, commensal infections (carriage) in the upper respiratory tract of clinically healthy individuals. If true, this would entail a radical revision of the general view of the natural history of leprosy. It would also provide an explanation for the observation that case finding and treatment programs have little demonstrable impact upon the future incidence of disease, and hence have major implications for our expectations concerning leprosy control.

Considering just the polymerase chain reaction (PCR) results, according to Table 1, the prevalence of PCR positivity was low overall (1.6%), and its trend by village was, if anything, exactly the opposite of the implied leprosy prevalence (the paper does not tell you this, but it is evident from the distribution of contacts that cumulative leprosy prevalence was highest in village 2 and lowest in village 3; whereas PCR prevalence was highest in village 3, lowest in village 2). From Table 2, PCR positivity seems to be, if anything, higher in non-contacts than in contacts (one might suppose that contacts would be older on average than non-contacts, but there is no discussion of this in the paper). From Table 3 we see no association between PCR status and BCG scar status. Leprosy status was assessed, but the paper says nothing about this beyond the comment that there were 42 cases in all, and that three were picked up in the baseline survey (we are not told in which villages). We are not told if the assays were carried out on the patients, let alone any results. We are not given an age distribution of the population, or of any of the other variables, but are told in the text that BCG scars were far more frequent among younger than older individuals (this is not surprising, as BCG is likely to have been introduced into the population in relatively recent years).

COMMENTARY
Is It Really M. leprae?1

1 This is a Commentary on an article by Cairns Smith, et al. published in this issue of the JOURNAL entitled “An Approach to Understanding the Transmission of M. leprae Using Molecular and Immunological Methods: Results from the MILEP2 Study,” which can be found on page 269.

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lence in follow ups of positives is the same as that in the initial survey, which could reflect either that PCR positivity is transient, or that the assay is at the limit of specificity, and is just picking up some other cross reacting nucleic acid in the population or in the laboratory. Other studies with this assay have experienced problems with its specificity (2), but this crucial issue is not dealt with explicitly here. The authors tell us that they discarded 16% of results because they failed quality control—but this statistic is not explained, and there is no discussion of its implications for the extent of false positives in the series reported (i.e., if x% of negative controls were assessed as positive in the laboratory, leading to discard of whole batches, and these manifest false positives were randomly distributed throughout the series, this proportion should have been subtracted from the proportion positive in their test series).

Concerning the IgA assay, two-thirds of all samples were considered to be positive “for mucosal responses to *M. leprae*.” We are shown no data by age, but are told in the text that “no difference was found between age groups.” This might be surprising, insofar as one might expect cumulative exposure to increase with age, but may be consistent with other evidence that IgA responses can be short-lived. The village distributions in Table 1 show little evidence of an association with leprosy contact status (villages 2 and 3 have highest and lowest proportions of contacts, respectively, but similar prevalences of IgA positivity). An association of IgA positivity with contact status is indicated in Table 2, but we are not told if the prevalence was higher in contacts of MB patients than in contacts of PB cases, or whether it was higher in contacts of recent compared to contacts of previous patients. Table 3 suggests higher IgA levels in individuals without BCG scar, compared to those with a scar. This may not make sense, unless we note that those without BCG scar are considerably older than those with a scar—and thus one might think the prevalence difference reflects age, and a higher IgA in older individuals. But we were told in the text there was no relation by age (though shown no data), so this relationship with absence of BCG poses a puzzle. There is something peculiar going on here, as the claim of no relationship of IgA with age implies a transient response (otherwise prevalence reflects cumulative incidence and must increase by age), whereas the absence of a BCG scar is unlikely to be a transient characteristic. I have no explanation for this! The most striking finding is shown in Table 5: an extraordinary, almost 10-fold, increase in prevalence of (strong) IgA positivity in the month of November. We are shown no evidence to indicate whether this was a once-off or a consistent phenomenon. Furthermore, this huge excess suggests that the month of collection is an important risk factor, and means that none of the other analyses can be interpreted without appropriate adjustment for the month of collection (i.e., the peculiar association with lack of BCG scar could be attributable to differential selection by age or BCG scar status during the November period). No such adjustments have been made.

What do these IgA data mean? Is it likely that two-thirds of all individuals were experiencing a short lived “mucosal response to *M. leprae*” at the time they were sampled? The IgA assay was performed with a crude whole *M. leprae* assay, and thus one would not expect it to be specific for *M. leprae* infection, but we are given no data on sensitivity or specificity (e.g., in currently infected MB patients, or in current contacts of MB patient). Cross reactivity is well known for T cell assays of different mycobacteria. The association with contact status may reflect some level of association of the assay with true *M. leprae* exposure, but further discussion of sensitivity and specificity, and detailed multivariate analysis is necessary to show convincingly whether any of the associations here reflects more than confounding.

Many readers will, like me, find this a tantalizing paper. They will struggle, and will wonder at the end of their effort if these data really do reflect *M. leprae*. I, for one, am not convinced that they do. The only internally consistent observation is the tendency for higher prevalence of both PCR and IgA positivity in the rainy season, though we have the irony of no association between the assays as shown in Table 4. Do the positive results reflect glimpses of *M. leprae*—or some other influence or artifact, perhaps some environmental mycobacterium? There is no way to tell from the data
presented here. Given the immense amount of work which went into the collection of these data, their complexity, and their susceptibility to confound, a more detailed presentation of the data and quality controls, including results on the cases and contact groups, detailed descriptions by age and contact status, and appropriate multivariate analysis, is called for.

If the hypothesis that *M. leprae* is a common, benign, transient resident of nasal cavities in endemic communities were correct, it would have major implications for our understanding of leprosy, and for expectations of control based upon case finding and treatment. But it remains an hypothesis, and criticisms similar to those above can be levelled at the other papers which have appeared on this subject (1). Given the importance of the issue at stake, it is important to test the hypothesis more rigorously than it has been to date. In addition to the analytical suggestions above, here are two proposals: first, in order to make results of such PCR-based studies convincing, a large number (appreciably greater than the reciprocal of the prevalence rate of carriage) of nasal swabs from a non-endemic control population should be blind-coded and randomly mixed with the test series before they are sent to the testing lab. The results on these should be reported explicitly, and the percentage positives in these samples should be subtracted from the percentages declared positive in the test series. Second, individual nasal swabs from healthy subjects in the same endemic community could be pooled and inoculated into susceptible animals (mouse footpads or armadillos). These suggestions are not trivial, and would be expensive. However, given the importance of the issue at stake, I can think of few better ways to further our understanding of leprosy at this point, and hope that some research group will take up the challenge.

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**REFERENCES**


CORRESPONDENCE

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

A Comparative Study Between 12 and 24-Dose Therapeutic Regimens for Multibacillary Leprosy Patients

ABSTRACT

A comparative study was made of the initial and final bacterial indices (BIs) of 213 MB leprosy patients who had been administered 12-dose (Group 1/128 patients) and 24-dose (Group 2/85 patients) World Health Organization multi-drug therapy to measure the effectiveness of both treatment regimens. All patients were evaluated at the beginning of treatment, at 12 months, and again after 24 months had elapsed. Decline in BI values and average BIs at 24 months were found to be similar for both groups. Moreover, no statistical difference between the two treatment regimens was found in the frequency rate of reaction.

RÉSUMÉ

Une étude comparant les index bactérioscopiques (IB) initiaux et finaux de 213 patients lépreux multibacillaires qui avaient reçu, soit le traitement de 12 doses (1er groupe de 128 patients), soit le traitement de 24 doses (2ème groupe de 85 patients) de la polychimiothérapie recommandée par l’OMS, fut entreprise afin d’évaluer l’efficacité de chaque traitement. Tous les patients furent évalués au début du traitement, après 12 mois, puis enfin après 24 mois. Les 2 groupes présentèrent des diminutions de valeurs d’IB et des IB moyens à 24 mois tout à fait similaires. De plus, il n’y eut pas de différence significative dans la fréquence des réactions immunopathologiques entre les 2 groupes.

RESUMEN

Se hizo un estudio sobre los índices bacteriológicos (IB) inicial y final en 213 pacientes MB tratados con 12 dosis (grupo 1/128) o 24 dosis (grupo 2/85 pacientes) de la poliquimioterapia recomendada por la OMS con objeto de medir la eficacia de ambos esquemas de tratamiento. Todos los pacientes fueron evaluados al inicio del tratamiento, y después a los 12 y 24 meses de haberlo concluido. Los dos grupos mostraron una disminución en sus IB y un IB promedio similar a los 24 meses post tratamiento. Tampoco se encontraron diferencias estadísticamente significativas entre las frecuencias de reacción observadas en los dos grupos de pacientes.

1Received for publication on 18 August 2003. Accepted for publication on 26 April 2004. Reprint requests to: Dr. Euzenir Nunes Sarno, Leprosy Laboratory, Oswaldo Cruz Foundation, Av. Brazil 4365, Manguinhos, Rio de Janeiro, RJ, Brazil, 21045-900. Email: euzenir@fiocruz.br
TO THE EDITOR:

In 1982, the World Health Organization (WHO) recommended administering a standard regimen of multi-drug therapy (WHO/MDT) composed of rifampicin, dapsone, and clofazimine to multibacillary (MB) leprosy patients for a minimum of 2 yrs (24 doses). At its seventh meeting, the WHO Expert Committee on Leprosy stated that while the 24-dose treatment for MB leprosy remained valid, the duration of MDT could be shortened to 12 months (5), which, having been well received by most of the of the major endemic countries, is now being implemented worldwide (6).

This study was carried out with patients in attendance at the Leprosy Outpatient Clinic of the Oswaldo Cruz Foundation (FIOCRUZ) in Rio de Janeiro, R.J., Brazil. A longitudinal study was designed to compare the 12-dose with the standard 24-dose MDT to ascertain bacterial index (BI) reduction, frequency of reaction, and patients’ disability grade (DG) in two groups of selected MB leprosy patients. Group 1 was composed of 128 MB leprosy patients who initiated the 12-dose MDT regimen between 1998 and 2000. Group 2 consisted of 85 MB patients who received the 24-dose regimen between 1995 and 1997. All patients were previously classified according to the Ridley and Jopling scale in addition to appropriate clinical and histopathological parameters.

As inclusion criteria, an MB leprosy patient was defined as any untreated leprosy patient with typical MB lesions whose BI, confirmed in slit-skin smears taken from 6 sites, was higher than 0. Each patient’s DG was evaluated by an experienced physical therapist and graded as 0, 1, or 2 according to WHO criteria. Both groups were evaluated with regard to their respective BIs and DGs at three specific times: at the beginning of treatment; after 12 doses (end of treatment for Group 1); and at 24 months. Reactive episodes were constantly monitored during the 12, 24, and 36-month periods. By way of standard procedure, all reactive patients were administered prednisone.

Statistical analysis was performed with EPI-INFO 6, 2S (CDC) and STATISTICA software. The results of the contingency tables were studied via the chi-square ($\chi^2$) test, considering a confidence level of 95%. Linear regression analysis was adopted to compare each participant’s initial BI with his/her post-24-month BI. A multiple regression analysis model was used to control the effect of the initial attributes of each case with respect to therapeutic regimen and final BI; and a generalized linear regression model was used to determine risk of reaction.

Patient classification as to clinical form was as follows: Group 1 (12 doses)—42 (32.8%) were classified as BB; 40 (31.3%) as BL; and 46 (35.9%) as LL. Group 2 (24 doses)—30 (35.3%) were BB; 30 (35.3%) were BL; and 25 (29.4%), LL. At diagnosis, the mean age was 37.0 and 38.3, respectively. Group 1 was 75% male and Group 2, 60%. The mean BI at the beginning of treatment was 2.27 for Group 1 and 2.64 for Group 2 (Table 1). Initial BIs were grouped as follows: BI = 0 to 1: 27.2% (Group 1)/25.9% (Group 2); BI = 1 to 2: 18.0%/14.1%; BI = 2 to 3: 21.9%/14.1%; BI = 3 to 4: 22.7%/23.5%; BI = 4 to 5: 9.4%/18.8%; and BI = 5 to 6: 0.85/3.5%.

The average BIs of both groups were sim-
ilar at the beginning of treatment (2.27 and 2.64, respectively), close again after 12 months (1.56 and 1.75, respectively), corresponding to discharge for Group 1 and the middle of treatment for Group 2; and again very close (1.03 and 1.06) after 24 months, which is also the 12-month period during which Group 1, still under surveillance, received no specific medication and Group 2 had just completed treatment (see Fig. 1). At 3.3% and 3.8%, respectively, no significant statistical difference was found between the average BI monthly reduction rate as a result of either the 12- or 24-dose treatment regimen. Moreover, since the regression coefficients of the two groups were similar and statistically significant, it was seen that initial BIs as predictors of final BIs did not depend on the number of doses administered. In fact, no association between treatment regimen and the BIs reported at 24 months could be found. As expected, however, a significant relationship between the BIs ascertained at 24 months and the initial and post-12-month BIs was demonstrated.

While the percentage of reaction was high in both groups (>60%), no significant statistical difference in the frequency rate of reaction was found between them. Furthermore, no change in disability grade occurred during the study (60% remained DG = 0).

Precisely how long the MB form(s) would best be treated to achieve maximum results remains controversial in view of the fact that a significant number of patients continued to be bacillary positive at the end of specific treatment (1-3) regardless of therapeutic scheme. From an operational standpoint, however, reducing the length of treatment without prejudicing its outcome has become a necessity in order to facilitate the implementation of MDT in distant rural areas where medical care is often scarce and/or inaccessible.

It is agreed that the time period covered by this study was insufficient to adequately evaluate the occurrence of relapses. To address this shortcoming, another study extending the follow-up periods for both Groups 1 and 2 for an additional 5 yrs is currently in progress.

Acknowledgment. We extend our thanks to Rita Maria de Oliveira Pereira, Denise Vieira de Moura, and Edson Araripe Albuquerque for their technical support throughout the study, as well as to Judy Gre- van for editing the text.

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![Figure 1](https://via.placeholder.com/150)

**Fig. 1.** This graph shows the average BI reductions measured at diagnosis, at 12, and at 24 months of both therapeutic regimens.


TO THE EDITOR:

Butyrylcholinesterase (BuChE) is a multifunctional enzyme (1, 8) mainly regulating cholinergic transmission, and is involved in growth, differentiation, and degeneration of nervous tissue (9). BuChE in the serum is a 90 kDa glycoprotein with three major subunits of approximately 50 kDa, 21 kDa and 20 kDa. The 50 kDa subunit has zinc-dependent peptidase activity at the carboxy terminus, and the 20 kDa subunit has distinct sites of butyrylcholinesterase and amine sensitive aryl acylamidase activities (2, 13, 14). BuChE in the skin, serum, and cerebrospinal fluid (CSF) are isoforms having similar peptidase and esteratic sites (5, 12, 15).

Plasma BuChE is found to be altered in chronic diseases like breast cancer and in Alzheimer’s disease (16, 17). Our earlier work has shown that there is an increased BuChE level in the nerves of leprosy-affected individuals (18). The present study was done to learn the pattern of BuChE in the serum of leprosy patients and compare it to normal controls.

Twenty-nine leprosy patients were included in the study; they included 8 borderline tuberculoid (BT) leprosy, 5 borderline lepromatous (BL), 5 lepromatous (LL), 4 pure neuritic leprosy (PNL), and 24 normal controls. Additionally 26 HIV-infected individuals were also studied. After informed consent, 2 ml of blood was collected from each patient. The serum was separated and stored at –20°C until the enzyme assay was carried out. The enzyme assay kit used was obtained from SPINREACT, S.A. Ctra. Santa Coloma, Spain. BuChE in serum was estimated by the method of Ellman, et al. (6) using butyrylcholine as substrate. In brief, 50 mmol/L phosphate buffer pH 7.7 containing 7 mmol/L butyrylthiocholine substrate and 0.25 mmol/L 5,5 dithio bis (2-nitrobenzoic acid) in a total volume of 500 µl was the reaction mixture, which was incubated with 4 µl of serum at 26°C. The optical density was measured at intervals of one minute spectrophotometrically at 405 nm. The calculation of BuChE activity in serum was according to instructions provided in the kit, where one unit of cholinesterase activity was defined as ΔE/60 sec ×11,355 = units/litre under the standard assay conditions (at 26°C). Statistical analysis was carried out using students “t” test.

The BuChE activity obtained in different categories of samples is presented in The Table. The mean values in all forms of leprosy, as well as HIV, were lower than the in normal sera. However, when each group was compared with the normal mean, the t value was not significant for any of the group except for PNL (p <0.1). When individual BuChE activity units were plotted in a scatter graph, each of the groups had quite a few patients showing a value less than the lowest normal value.

A decrease of serum cholinesterase has been suggested to be associated with susceptibility to leprosy and particularly to the tuberculoid and lepromatous forms of the disease (3, 11, 19). In the present study, however, there were no statistically significant differences observed between the different forms of disease (Genetic variation BuChE has not been taken into consideration in this investigation). The scatter of values observed in each group indicate that it may not be a specific marker for any particular form of leprosy. Other studies have found that during exacerbations of leprosy there is an abrupt decrease in serum cholinesterase levels, which could be attributed to liver-specific granulomatous change and parenchymal cell damage (4).

Leprosy is an infectious disease that primarily affects the nerves. Nerve damage is an important and dreaded complication of the disease. In our earlier report an increase of BuChE levels in the nerves of leprosy

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patients was observed (18), whereas an insignificant alteration was observed in serum BuChE levels. This suggests that an increase in the nerve could be due to the highly localized nature of the nerve destruction in leprosy and predilection of the *M. leprae* to peripheral nerve.

The reported BuChE decrease in skin tissue (7), increase in nerve BuChE (18), and the present observation of plasma BuChE indicates that it is a multifunctional enzyme with different expressions in different tissues (12,15). Cholinesterase activity has been found to be altered in cerebrospinal fluid and erythrocytes of patients with Alzheimer’s disease (17). Altered cholinesterase activity and glycosylation of AchE have also been found in human breast cancer (16).

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False Positive Reaction of the Immunohistochemistry Technique Using Anti-BCG Polyclonal Antibodies to Identify Mycobacterium leprae in Wild Nine-Banded Armadillos

ABSTRACT

The authors studied 66 wild nine-banded armadillos from Brazil. The ear samples were collected and Ziehl-Neelsen or Fite-Faraco stains were performed, as well as immunostaining using polyclonal BCG antibody, to evaluate the presence of the Mycobacterium leprae. The AFB were not detected by the Ziehl-Neelsen or Fite-Faraco staining, neither immunexpression of the BCG marker. However, many normal structures from the ears of the nine-banded armadillos, such as condrocytes, condroblasts, fibroblasts and endothelial cells, and Gram positive bacteria cocci, showed false positive reaction by the BCG marker. The authors discuss the use of the immunohistochemical studies with the polyclonal BCG antibody to identify M. leprae antigens in wild armadillos.

RÉSUMÉ

Les auteurs ont étudié 66 tatous à neuf bandes sauvages du Brésil. Les échantillons d’oreille furent prélevés et des colorations de Ziehl-Neelsen et de Fite-Faraco, ainsi qu’un immunomarquage utilisant un sérum polyclonal dirigé contre le bacille de Calmette et Guérin (BCG), afin d’évaluer la présence de Mycobacterium leprae. Des bacilles acido-résistants (AAR) ne furent pas détectés par le Ziehl-Neelsen ou le Fite-Faraco et une immun-expression du marqueur BCG ne fut pas observée non plus. Cependant, de nombreuses structures normales des oreilles de tatous à neuf bandes, telles que des chondrocytes, des chondroblastes, des fibroblastes et des cellules endothéliales, ainsi que des coques bactériennes positives à la coloration de Gram, ont montré une réaction faussement positive au marqueur BCG. Les auteurs discutent de l’utilité des études immunohistochimiques avec un anticorps polyclonal dirigé contre le BCG pour identifier des antigènes de M. leprae chez les tatous sauvages.

RESUMEN

Se estudiaron 66 armadillos silvestres de 9 bandas de Brasil. Se colectaron muestras de las orejas, se seccionaron y se tiñeron por Ziehl-Nelseen o Fite-Faraco, y se evaluó la presencia de Mycobacterium leprae utilizando un anticuerpo policolonal anti-BCG. No se detectaron bacilos ácido-resistentes por las tinciones de Ziehl-Nelseen/Fite-Faraco y tampoco se encontraron bacilos con el suero anti-BCG. Sin embargo, se observó que muchas estructuras normales de las orejas de los armadillos, tales como condrocitos, condroblastos, fibroblastos y células endoteliales, y bacterias Gram negativas, dieron reacciones falsas-negativas con el reactivo anti-BCG. Los autores discuten el uso de los estudios inmunohistoquímicos con el suero policolonal anti-BCG para identificar antígenos de M. leprae en los armadillos silvestres.

TO THE EDITOR:

Current identification of Mycobacterium leprae is done by Fite-Faraco or Ziehl-Neelsen stains, but low numbers of bacillus in the tissue could make identification difficult. Some authors have reported that immunohistochemistry techniques demonstrate antigens of M. leprae instead of the whole bacillus, increasing the sensibility of the histopathology diagnose mainly in paucibacillary forms (1,5,7). Takahashi, et al. (7) identified more than 20% of the AFB from of the skin biopsy of the indeterminate leprosy (paucibacillary form) using immunostaining with anti-BCG (bacilli of Calmette-

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Guérin) antibodies. Using the same technique, Barbosa Jr., et al. (1) found acid-fast bacilli (AFB) in 50% of the skin biopsies where they did not find AFB before by Fite-Faraco staining. The antigens were detected in several sites of the dermis, mainly in the perivascular region. Kutzner, et al. (4) described the anti-BCG immunostaining technique as a promising screening tool for the detection of common infectious microorganisms in humans. The anti-BCG antibodies do not react with the normal structures from human skin nor with inflammatory cells, and produce little background, although Schettini, et al. (6) observed that melanophages and mast cells could be stained. Some authors have demonstrated cross reaction between polyclonal antibodies and a large spectrum of fungi and bacteria (2, 3, 4, 9). Bonenberger, et al. (2) established that some microorganisms such as Mycobacteria and Nocardia were strongly stained by immunostaining with anti-BCG; Criptococcus, Pythium and Prototheca were partially stained; spirochetes and protozoa (Leishmania) were not stained.

We analyzed 66 wild nine-banded armadillos, Dasypus novemcinctus species, from the State of Espírito Santo, localized in Southern region of Brazil. The purpose of this research was to evaluate if im-
immunostaining with a polyclonal anti-BCG antibody was an appropriate diagnostic tool for \textit{M. leprae} detection in these animals. After the armadillos had been captured, the ears, margins were collected and fixed in 10% neutral buffered formalin.

The paraffin-embedded samples were sectioned and slides were stained with Ziehl-Neelsen or Fite-Faraco stains and immunohistochemistry. We used the immunostaining with the anti-BCG on human positive-control biopsies from multibacillary patients, to compare with the samples from the armadillos. After blocking of endogenous peroxidase, the slides were incubated with the polyclonal rabbit antibody (anti-\textit{Mycobacterium bovis}, DAKO A/S, Denmark), Dilution of 1:100,000 was done in PBS buffered pH 7.4 with bovine serum albumin (BSA). After this, the slides were incubated with the secondary antibody, biotinylated goat anti-mouse/rabbit Ig (StrepABComplex/HRP Duet mouse/rabbit, DAKO A/S, Denmark), diluted 1:200 in PBS and with the strepavidin-Biotin-peroxidase complex 1:200 in PBS. The antigens were visualized after incubation with the diaminobenzidine tetrahydrochloride 60 mg% (SIGMA, EUA), DMSO 1 ml, 6% H\textsubscript{2}O\textsubscript{2} 20 volume and PBS 100 ml for five minutes at room temperature. Negative controls were prepared by omission of the primary antibody and substitution with BSA. The readings were carried out on coded, “blinded” slides.

The histopathological examination of the ear biopsies showed an inflammatory infiltrate of the lympho-histiocytes and neutrophils, but no AFB was found by Ziehl-Neelsen or Fite Faraco staining, or by immunohistochemical study. However, nuclei of many normal cells, such as condrocytes, condroblasts, fibroblasts, endothelial cells (Fig. 1), and Gram positive bacteria cocci produced immunostaining with the anti-BCG marker (Fig. 2). In all the material, the primary antibody was replaced by BSA to demonstrate that there are no reactions due to the secondary antibody or slide background. The human controls showed macrophages immunostained with the anti-BCG marker (Fig. 3).

The immunohistochemical technique using polyclonal anti-BCG antibody for detection of \textit{M. leprae} antigens in ears from
wild armadillos, or other armadillo tissues, has not been reported before. We found several structures presenting immunoperoxidase to the anti-BCG marker in the epidermis and the dermis of the ear biopsies, without the morphologic features presented by the *M. leprae* immunostained antigens previously described. Probably in wild nine-banded armadillo, microorganisms other than *Mycobacteria* could be responsible for false positive results in these tissues. These findings agree with previous reports (2-4). Another interesting feature observed is that the polyclonal anti-BCG antibody stains Gram positive bacteria. Some animals showed very similar structures to the morphology of coccus. This similarity can create doubt about the use of the immunohistochemical methods using the polyclonal anti-BCG alone to detect the *M. leprae* infection in wild armadillos. We do not know if this technique would be useful to detect mycobacterial antigens in tissue other than ears, such as skin, lymphnodes, or liver. In conclusion, the immunohistochemical technique, using anti-BCG antibodies, is a very useful tool for the diagnosis of human paucibacillary or indeterminate forms of leprosy, but in wild nine-banded armadillos immunostaining of normal structures of the ear and cross reaction with different infectious agents could difficult its utilization for Mycobacterial antigen detection difficult.

**Acknowledgment.** The authors would like to thank Dr. Carlos Faria from Santa Casa de Misericordia Medical School, Vitória, Brazil; Dr. Fausto Edmundo Lima Pereira from Federal University of Espirito Santo, Vitória, Brazil for precious critical suggestions; Dr. Charles K. Job from the Department of Pathology, St. Thomas Hospital and Leprosy Center, Chettupattu, India for the histopathologic review, and Ms. Suely Nonogaki from Adolpho Lutz Institute, São Paulo, Brazil for technical assistance.

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The authors studied 66 wild nine-banded armadillos from Brazil. The ear samples were collected and Ziehl-Neelsen or Fite-Faraco stains were performed, as well as immunostaining using polyclonal BCG antibody, to evaluate the presence of the *Mycobacterium leprae*. The AFB were not detected by the Ziehl-Neelsen or Fite-Faraco staining, neither immunexpression of the BCG marker. However, many normal structures from the ears of the nine-banded armadillos, such as condrocytes, condroblasts, fibroblasts and endothelial cells, and Gram positive bacteria cocci, showed false positive reaction by the BCG marker. The authors discuss the use of the immunohistochemical studies with the polyclonal BCG antibody to identify *M. leprae* antigens in wild armadillos.

**RÉSUMÉ**

Les auteurs ont étudié 66 tatous à neuf bandes sauvages du Brésil. Les échantillons d’oreille furent prélevés et des colorations de Ziehl-Neelsen et de Fite-Faraco, ainsi qu’un immunomarquage utilisant un sérum polyclonal dirigé contre le bacille de Calmette et Guérin (BCG), afin d’évaluer la présence de *Mycobacterium leprae*. Des bacilles acido-résistants (AAR) ne furent pas détectés par le Ziehl-Neelsen ou le Fite-Faraco et une immuno-expression du marqueur BCG ne fut pas observée non plus. Cependant, de nombreuses structures normales des oreilles de tatous à neuf bandes, telles que des chondrocytes, des chondroblastes, des fibroblastes et des cellules endothéliales, ainsi que des coques bactériennes positives à la coloration de Gram, ont montré une réaction faussement positive au marqueur BCG. Les auteurs discutent de l’utilité des études immunohistochimiques avec un anticorps polyclonal dirigé contre le BCG pour identifier des antigènes de *M. leprae* chez les tatous sauvages.

**RESUMEN**

Se estudiaron 66 armadillos silvestres de 9 bandas de Brasil. Se colectaron muestras de las orejas, se seccionaron y se tintieron por Ziehl-Neelsen o Fite-Faraco, y se evaluó la presencia de *Mycobacterium leprae* utilizando un anticuerpo polyclonal anti-BCG. No se detectaron bacilos ácido-resistentes por las tinciones de Ziehl-Nelseen/Fite-Faraco y tampoco se encontraron bacilos con el suero anti-BCG. Sin embargo, se observó que muchas estructuras normales de las orejas de los armadillos, tales como condrocitos, condroblastos, fibroblastos y células endoteliales, y bacterias Gram negativas, dieron reacciones falsas-negativas con el reactivo anti-BCG. Los autores discuten el uso de los estudios inmunohistoquímicos con el suero polyclonal anti-BCG para identificar antígenos de *M. leprae* en los armadillos silvestres.
Guérin) antibodies. Using the same technique, Barbosa Jr., et al. (1) found acid-fast bacilli (AFB) in 50% of the skin biopsies where they did not find AFB before by Fite-Faraco staining. The antigens were detected in several sites of the dermis, mainly in the perivascular region. Kutzner, et al. (4) described the anti-BCG immunostaining technique as a promising screening tool for the detection of common infectious microorganisms in humans. The anti-BCG antibodies do not react with the normal structures from human skin nor with inflammatory cells, and produce little background, although Schettini, et al. (6) observed that melanophages and mast cells could be stained. Some authors have demonstrated cross reaction between polyclonal antibodies and a large spectrum of fungi and bacteria (2, 3, 4, 9). Bonenberger, et al. (2) established that some microorganisms such as Mycobacteria and Nocardia were strongly stained by immunostaining with anti-BCG; Cryptococcus, Pythium and Prototheca were partially stained; spirochetes and protozoa (Leishmania) were not stained.

We analyzed 66 wild nine-banded armadillos, Dasypus novemcinctus species, from the State of Espírito Santo, localized in Southern region of Brazil. The purpose of this research was to evaluate if im-

Fig. 1. Immunohistochemistry findings in ear fragment. Nuclei of the condrocytes, condroblasts, fibroblasts, endothelial cells presenting immunoreactivity to the anti-BCG marker (Objective 40×).

Fig. 2. Immunohistochemistry findings in ear fragment. Inflammatory infiltration of the lymphohistiocytes and neutrophils, and Gram positive bacteria as coccus presenting immunoreactivity to the anti-BCG marker (Objective 20×).
munnostaining with a polyclonal anti-BCG antibody was an appropriate diagnostic tool for *M. leprae* detection in these animals. After the armadillos had been captured, the ears, margins were collected and fixed in 10% neutral buffered formalin.

The paraffin-embedded samples were sectioned and slides were stained with Ziehl-Neelsen or Fite-Faraco stains and immunohistochemistry. We used the immunostaining with the anti-BCG on human positive-control biopsies from multibacillary patients, to compare with the samples from the armadillos. After blocking of endogenous peroxidase, the slides were incubated with the polyclonal rabbit antibody (anti-*Mycobacterium bovis*, DAKO A/S, Denmark), Dilution of 1:100,000 was done in PBS buffered pH 7.4 with bovine serum albumin (BSA). After this, the slides were incubated with the secondary antibody, biotinylated goat antirabbit Ig (StrepABComplex/HRP Duet mouse/rabbit, DAKO A/S, Denmark), diluted 1:200 in PBS and with the strepavidin-Biotin-peroxidase complex 1:200 in PBS. The antigens were visualized after incubation with the diaminobenzidine tetrahydrochloride 60 mg% (SIGMA, EUA), DMSO 1 ml, 6% H$_2$O$_2$, 20 volume and PBS 100 ml for five minutes at room temperature. Negative controls were prepared by omission of the primary antibody and substitution with BSA. The readings were carried out on coded, “blinded” slides.

The histopathological examination of the ear biopsies showed an inflammatory infiltrate of the lympho-histiocytes and neutrophils, but no AFB was found by Ziehl-Neelsen or Fite-Faraco staining, or by immunohistochemical study. However, nuclei of many normal cells, such as condrocytes, condroblasts, fibroblasts, endothelial cells (Fig. 1), and Gram positive bacteria cocci produced immunostaining with the anti-BCG marker (Fig. 2). In all the material, the primary antibody was replaced by BSA to demonstrate that there are no reactions due to the secondary antibody or slide background. The human controls showed macrophages immunostained with the anti-BCG marker (Fig. 3).

The immunohistochemical technique using polyclonal anti-BCG antibody for detection of *M. leprae* antigens in ears from...

**Fig. 3.** Immunohistochemistry findings in human skin fragment from leprosy multibacillary patient, the human positive control (Objective 40x).
wild armadillos, or other armadillo tissues, has not been reported before. We found several structures presenting immunexpression to the anti-BCG marker in the epidermis and the dermis of the ear biopsies, without the morphologic features presented by the *M. leprae* immunostained antigens previously described. Probably in wild nine-banded armadillo, microorganisms other than *Mycobacteria* could be responsible for false positive results in these tissues. These findings agree with previous reports (2-4). Another interesting feature observed is that the polyclonal anti-BCG antibody stains Gram positive bacteria. Some animals showed very similar structures to the morphology of coccus. This similarity can create doubt about the use of the immunohistochemical methods using the polyclonal anti-BCG alone to detect the *M. leprae* infection in wild armadillos. We do not know if this technique would be useful to detect mycobacterial antigens in tissue other than ears, such as skin, lymphnodes, or liver. In conclusion, the immunohistochemical technique, using anti-BCG antibodies, is a very useful tool for the diagnosis of human paucibacillary or indeterminate forms of leprosy, but in wild nine-banded armadillos immunostaining of normal structures of the ear and cross reaction with different infectious agents could difficult its utilization for Mycobacterial antigen detection difficult.

**Acknowledgment.** The authors would like to thank Dr. Carlos Faria from Santa Casa de Misericordia Medical School, Vitória, Brazil; Dr. Fausto Edmundo Lima Pereira from Federal University of Espírito Santo, Vitória, Brazil for precious critical suggestions; Dr. Charles K. Job from the Department of Pathology, St. Thomas Hospital and Leprosy Center, Chettuppattu, India for the histopathologic review, and Ms. Suely Nonogaki from Adolpho Lutz Institute, São Paulo, Brazil for technical assistance.

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Doug’s contribution in this field was enormous, and largely unrecognized. His meticulous reports were accompanied by stained slides that were posted back to those who had submitted each biopsy, so that they could learn from the microscopic findings in their own patients. As a teacher, he was in a class of his own; kindly, endlessly helpful, slow to criticize and manifestly well informed about all aspects of this difficult specialty.

Following his death in December 2003, the family and many friends came to the Eltham United Reformed Church for a service of thanksgiving. In her moving address, the Reverend Margaret Juhasz quoted the words of one of her predecessors, Ronald Ward: “Nobody could know Douglas well without being aware of a certain

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fineness in his personality. This isn’t quite the word, but I can’t think of a better. His integrity and sensitivity were there for all to see, as was his quiet sense of humor, which I found most endearing. Quiet is a good word for him. His gentle voice and manner reflected a tranquility within which was the fruit of a steady faith. I am sure it is this which helped him through all the blows and disappointments of life.”

Our sympathy goes to Christopher, Roderick, Patricia, Sheila and Anthony, and to all those in the mission and medical fields who had the privilege of knowing him.

—A. Colin McDougall
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—A. Colin McDougall

*Honorary Consultant*

*Department of Dermatology*

*The Churchill Hospital*

*Oxford OX3 7LJ, UK*
Emanuel Faria was born in British Guiana on Christmas Day, 1912. His mother, originally from Portugal, clearly hoped for great things for her son on whom she had bestowed such a powerful name—Emanuel Jesus. However, like so many others whose dreams have been cut short by a diagnosis of leprosy, Emanuel’s life was to take turns that no one could have foreseen.

In 1927, at the age of 15, Emanuel was admitted to the Mahaica Leprosy Hospital outside of Georgetown, British Guiana, and spent the next five years of his life there. After being treated with a compound of chaulmoogra oil and creosote, he was discharged and returned home to work in his father’s business. However, after a period of years, he returned to Mahaica due to increasing problems with his hands.

In 1968, Emanuel felt the need to leave what was then Guyana for political reasons and also hoped to have reconstructive surgery at Carville, where he arrived that same year. He already knew about The Star from correspondence with Stanley Stein and in 1969 started to work as a proofreader. He would later say: “My philosophy of life is that we all have to contribute to the pool of good. We cannot expect others to contribute while we just go and draw from it and don’t put anything into it. When I was given the opportunity to work at The Star, I felt I was contributing something.”

At Carville, Emanuel had the opportunity to meet recognized authorities in the field of leprosy from different countries and developed the feeling that The Star, which had previously focused largely on social and human rights issues, needed to have a medical component. He undoubtedly also saw this as a way in which to increase the readership of The Star which, by 1990, was sent to more than 120 countries throughout the world.

Adding a medical component to The Star also enabled Emanuel to fulfill, in a small way, a part of his life that had been taken from him. He would later say: “If I didn’t come down with this disease, I would be in England. I believe I would have studied medicine. I would have tried to win the Guyana Scholarship and either gone to Oxford or Cambridge.”

Emanuel served on the Board of Directors of American Leprosy Missions for three terms, a total of nine years. He became the Editor of The Star in the mid-1990’s and worked diligently to keep it alive through periods of major uncertainty, change and the ultimate decline in the population of Carville. Yet, despite uncertainties about his own future that weighed heavily on his mind in his final years, Emanuel always put The Star first. Indeed, this man of extreme intelligence who read ancient Greek history and poetry by Percy Bysshe Shelley in his spare time, knew that the voice of The Star and what it represents to so many individuals throughout the world, must continue.
News of his death on March 17, 2004, brought many tributes, including one from Miyoji Morimoto in Japan: “His consistent appeal for the actualization of a society without discrimination and prejudice against persons who have been affected by Hansen’s Disease and the restoration of their dignity, has reached out to the world. . . . His teaching will abide. . . . May his soul rest in peace.”

—Anwei Skinsnes Law

EMANUEL JESUS FARIA

Not unlike Father Damien and Stanley Stein, Emanuel Faria was a man with a mission in life. Emanuel Jesus Faria began his life in Georgetown, Guyana, and learned at an early age that self determination was his best direction in life.

Emanuel came to the United States in hopes of seeking citizenship and a better opportunity for a better way of life. He arrived in 1969.

Mr. Faria was a self-educated man, with a great love of history. His most prized possession was the American Flag he received when he became an American Citizen. For all official photographs that were to appear in the press, he always requested that his American Flag be included in the photograph. Emanuel stated on one occasion that he always admired the United States and felt his arrival in the United States was the fulfillment of a life-long dream. He was a student and professional advocate of freedom and human rights.

So what better qualifications for a writer/editor of The Star Magazine, a publication dedicated to radiating the light and truth of Hansen’s Disease worldwide.

Emanuel became a staff writer, and worked his way up the editorial line, to become Editor of the Star magazine in Carville, Louisiana, USA. He gave the old Star magazine a renewed future by bringing the publication into the digital age through online access worldwide.

Emanuel was intelligent, conscientious, and hard working, an individual who continued to educate himself throughout his life. He was a quiet man, who began his Star career as a staff writer and became Editor of the International Magazine in the 1990’s. In 2001, he brought the Star magazine into the digital age. This meant learning to navigate the internet and computer during his 90th venue of life. He rose to the task at hand with a sense of journalistic accomplishment not shared by many in the publishing world.

Simply because of his forward progressive thinking, anyone worldwide can now access the Star online, and the magazine can be published from any location, not just from behind the Gates of Carville.

He was a member of the American Leprosy Missions Board of Directors for many years and through his dedication and generosity to those less fortunate than himself, provided scholarly and monetarily of himself. He was dedicated to making this a better world for all. He was an activist in the fight for integration, dignity and economic advancement. (I.D.E.A.) His portrait was on display in the United Nation in New York, during the celebration, Quest for Dignity in 1997. He was an honest man with simple tastes.

Quoting from the IDEA newsletter a poem by Chen Shi Yun:

“Dying Wood Meets Spring”
The ill tree is dying,
Attacked by wind and rain
Yet lucky to meet spring and be
nourished
The dying tree is dreaming of a new life.

Throughout history and into the present, individuals affected by specific diseases have been marginalized by society, denied their identities and their most basic human rights. Whole families have been stigmatized because of society’s attitudes toward some diseases, that have generally been based on folklore, cultural traditions and outdated images rather than facts.

Yet, throughout history, there have also been, many powerful examples of individuals who have refused to be labeled by society. Men and women have risen their voices, sometimes in necessary protest, but
more often in poetry, music and art, to defy society’s stereotypes, labels and limitations. Emanuel was one such internally liberated individual. A free soul liberated only in his mind and work.

Emanuel’s life was a voice of humanity, not a meek voice, but a powerful strong voice for all people worldwide, that have suffered the stigma of man’s inhumane fears and rejections. He was a crusader who dispelled ignorance, prejudices and superstition surrounding a disease. Emanuel “the man” quiet, intellectual, self-educated, dedicated and a sincere gentleman. Emanuel had courage and refused to quit until the battle with ignorance was won. He was working on the coming issue of The Star, when he became ill and went into the hospital, where he spend his last few days. His life was both an example and inspiration to others. He was a serious student of history and life, with a sincere work ethic. He was his own man and was spoken of often, as quite a character.

Emanuel played the role of Stanley Stein in the television documentary, “Exiles in our own Country.” Over the years as writer for The Star Magazine he conducted interviews with foreign and national physicians and allied health professionals, as well as many correspondents and news media journalist. He wrote numerous articles on the progress of medical research. He spoke eloquently to medical groups or individuals, just interested in The Star. Medical writer, orator, actor, editor and United States citizen, describes briefly our friend. His leadership, perseverance, professional style, and smile, will be missed.

Emanuel was intelligent, conscientious, hard working and an individual who continued to educate himself throughout his life. In 2001, he brought The Star Magazine into the digital age. This meant learning to navigate the internet and computer with failing sight, but not insight. As an elder in our community, his life experiences should remind us all of our gifts, and how we might use these gifts to create, educate and contribute to our society in our own way.

Emanuel rose to the task when asked, not only to learn the computer, but to publish The Star online. Nearing his 90th birthday, he began to embrace the computer, internet and the digital age wholeheartedly. Emanuel was a true lover of history and knowledge and found a new world of exposure in his computer. I remember him saying, “can you really get the BBC!”

Father Damien, Mary Ruth, Stanley Stein, Dr. Paul Brand, and so many others, join Emanuel in helping us continue to make this a more humane world of hope, understanding and dignity for all people.

Thank you, Emanuel, from all the patients worldwide and many others, working around the world to eliminate the stigma of diseases, share accurate knowledge of progress, and promote educated truths. The world is a better place because you were an active player on the great stage of life. Your friends will greatly miss you and are grateful for the time shared together.

—Tanya Thomassie

National Hansen’s Disease Programs
Media/Public Affairs
After a long illness, lovingly nursed by his family and many carers, “Doug” Harman died at home in December 2003. He was born in Ramsgate, Kent, in 1915 and educated at Chatham House School, followed by medical studies in Edinburgh University. After marriage to Gladys in 1939, they embarked on a journey to China to work with the London Missionary Society (now the Council for World Mission). Following language studies in Amoy, they went to a small mission hospital in Changchow in the province of Fukien. Three children were born there, Chris, Rod and Pat. By 1944, the Japanese war against China became so dangerous that the family had to leave, trekking through China and eventually by air into India, over mountainous terrain, ravines and rivers, subjected to bombing and often short of food.

After a period of furlough in Britain, they returned to China in 1946 and worked there until 1950, during which time the family increased with the birth of Sheila and Tony. Doug later spent a period with the LMS in Zambia (previously Northern Rhodesia), where he further developed his interest in the management and treatment of leprosy. In 1956, he was seconded to The Leprosy Mission in Hong Kong, based on the island of Hay Ling Chau. Although not trained as a surgeon, he learned to operate on hands damaged by leprosy, using techniques pioneered by Dr. Paul Brand, whose work in this field had already gained international recognition. In 1960, Doug returned home and from 1961 until his retirement in 1980, worked at the Leprosy Study Center in London, mainly on the histopathological changes in the tissues of leprosy patients from many parts of the world.

By nature modest and retiring about his experiences and accomplishments, he nevertheless contributed a remarkable account of the work of the public health nurses of the Changchow Union Hospital (Conquest by Healing, Volume XXVII, No. 1, March 1952), including the building of a new hospital, the setting up of immunization clinics and the development of mobile clinic work in neighboring villages. The epic story of their heroic trek from the Sino-Japanese war into India and then back to Britain has yet to be written, but it is clear testimony to the immense determination of Doug and his family, backed by their deep Christian faith.

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—A. Colin McDougall
Honorary Consultant
Department of Dermatology
The Churchill Hospital
Oxford OX3 7LJ, UK
NEWS and NOTES

This department furnishes information concerning institutions, organizations, and individuals engaged in work on leprosy and other mycobacterial diseases, and makes note of scientific meetings and other matters of interest.

Calendar of Meetings and Events

<table>
<thead>
<tr>
<th>Day</th>
<th>mm/yy</th>
<th>Location</th>
<th>Details</th>
<th>Contact</th>
<th>E-mail</th>
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<tbody>
<tr>
<td>26–30</td>
<td>9/4/2004</td>
<td>Sydney</td>
<td>Joint Meeting of the New Zealand and Australian Societies for Microbiology</td>
<td>Australian Society for Microbiol.</td>
<td><a href="mailto:admin@theasm.com.au">admin@theasm.com.au</a></td>
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<tr>
<td>29–2</td>
<td>Sep/Oct-04</td>
<td>Tehran</td>
<td>7th International Congress of Dermatology</td>
<td>Yahya Dowlati</td>
<td><a href="mailto:info@irander.org">info@irander.org</a></td>
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<tr>
<td>13–8</td>
<td>Sep/Oct-04</td>
<td>Ethiopia</td>
<td>“ALERT Training Program in Tuberculosis, Leprosy and Research Methodology”</td>
<td>ALERT Training Division</td>
<td><a href="mailto:leprosytb@telecom.net">leprosytb@telecom.net</a></td>
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<tr>
<td>30–4</td>
<td>Sep/Oct-04</td>
<td>Boston</td>
<td>Infectious Disease Society of America</td>
<td>Infect. Dis. Soc. of America</td>
<td><a href="mailto:info@idsociety.org">info@idsociety.org</a></td>
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<tr>
<td>11–22</td>
<td>10/4/2004</td>
<td>Ethiopia</td>
<td>“ALERT Training Program in Tuberculosis, Leprosy and Research Methodology”</td>
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<tr>
<td>7–11</td>
<td>11/4/2004</td>
<td>Miami</td>
<td>53rd Annual Meeting of the American Society of Tropical Medicine &amp; Hygiene</td>
<td>ASTMH</td>
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<tr>
<td>1–5</td>
<td>12/4/2004</td>
<td>Bangkok</td>
<td>9th Western Pacific Congress on Chemotherapy and Infectious Diseases</td>
<td>Congress Secretariat</td>
<td><a href="mailto:wpccid2004@idthai.org">wpccid2004@idthai.org</a></td>
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<td>31–1</td>
<td>Jan/Feb-05</td>
<td>Midrand, S. Africa</td>
<td>African Leprosy Congress</td>
<td>Peter Laubscher</td>
<td><a href="mailto:peter@tlm.co.za">peter@tlm.co.za</a></td>
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International Journal of Leprosy
Business Office
1 ALM Way, Greenville, SC 29601

2005 Membership and Subscription rates

The Officers and Council of the International Leprosy Association have, in accordance with the Constitution and By-laws of the organization, agreed on the following new rates for Membership of the ILA and subscription to the JOURNAL, applicable from January 1st, 2005.

The possibility of making the JOURNAL accessible online only, has been widely discussed, but such a policy would currently limit access quite severely in some parts of the world. We have therefore arranged with the publisher, Allen Press, for the continued quarterly printing of the JOURNAL for those Members and Subscribers who still require it.

<table>
<thead>
<tr>
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<tr>
<td>Individual membership of the ILA with full access to the online JOURNAL and the Members’ area of the ILA site, but no printed copy of the JOURNAL . . . USD 25.00</td>
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<tr>
<td>Individual membership of the ILA with full access to the online JOURNAL and the Members’ area of the ILA site, plus a printed copy of the JOURNAL . . . USD 100.00</td>
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<tr>
<td>Institutional subscriptions to the JOURNAL, with online access and a quarterly, printed copy of the JOURNAL . . . . . . . . . . . . . . USD 180.00</td>
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<tr>
<td>While there is open access to the online JOURNAL at present (please go to <a href="http://www">www</a>. leprosyjournal.org), from January, 2005, ac-</td>
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</table>
access to both the current and past issues will require a valid username and password, obtainable through the Business Office: ijl@leprosy.org. Institutional subscribers may arrange for direct access for specific computer IP addresses.

The ILA’s web-site is at www.leprosy-ila.org

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

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

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

The recipients will have been active members in their community for at least five years and will be champions for the cause of leprosy through their achievements. Each Award includes a prize of £1000 and a presentation plaque. The recipients will be invited to travel to Dublin, with a supporter of their choice, to receive the Award at an awards ceremony in the first week of June 2005.

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

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

Each award includes a prize of £1000 and a presentation plaque. The recipient will be invited to travel to Dublin for a special awards ceremony in the first week of June 2005. Nominations must be submitted on the official form available from Glynis Forbes. The deadline for nominations is 10 January 2005.


The International Leprosy Association is planning a regional African Leprosy Congress at Midrand north of Johannesburg, South Africa from 31st January to 3rd February 2005.

The Congress will bring together leprosy workers and scientists fighting the disease in Africa and from elsewhere. The purpose is to exchange information both scientific and operational, to accelerate progress towards the elimination of the disease and to ensure that the legitimate needs of leprosy-affected persons are met both in humanitarian and rehabilitation terms. The congress is planned to be co-organized by the government of South Africa and co-sponsored by the World Health Organization [WHO] and the International Federation of Anti-Leprosy Associations [ILEP].

Conference Details.
• Dates: 31st January through 3rd February 2005
• Venue: Eskom Convention Centre, Midrand, north of Johannesburg, South Africa
• Languages: English, French, and Portuguese-Interpretation services will only be available during the plenary sessions
• Objectives
  (i) To bring together leprosy workers from Africa and elsewhere to discuss technical and scientific issues including those relating to progress in leprosy control and elimination on the continent
  (ii) To exchange information and experiences
  (iii) To increase the commitment and motivation of participants toward anti-leprosy work
• Format: Invited lectures, symposia, free presentations [Oral and Poster]
Number of papers expected in open sessions: 75
Expected number of poster presentations: 50

- Sessions
Six open sessions for free presentations covering Epidemiology, Control and Elimination, Clinical aspects, Rehabilitation and Basic Sciences.
Three invited lectures covering Molecular Biology, Prevention of Deformities and Leprosy Elimination.
Three symposia covering changing leprosy scenarios in Africa, Reaching the Unreached and Social and Rehabilitation issues.

- Registration
Closing date for registration: 29th October 2004
Closing date for receipt of abstracts: 29th October 2004
Closing date for late registration: 31st December 2004

- Registration package
  - Four nights’ economy accommodation
  - All meals for four days: Commencing with Dinner on 30th January and ending with lunch on 3rd February 2005. Lunch will be provided for delegates who arrive on early morning flights at Johannesburg International Airport on 30th January
  - Transport to and from Johannesburg International Airport
  - Registration kit
  - Documentation

All other costs such as phone calls and bar service are for the personal account of the delegate.

Prospective participants are requested to register well in advance as accommodation is limited. Most guests will be accommodated in economy rooms which shared bathrooms, but a few en-suite rooms are available and will be allocated to those guests who book first.

- Registration fees
  a. For delegates from outside the Republic of South Africa
     ii. Late: US $ 600: US $ 100 non-refundable deposit payable by 31st December 2004, balance payable by 30th January 2005
     iii. Onsite registration: US $ 700
  b. Delegates from within the Republic of South Africa
     i. Normal: ZAR 2 300: ZAR 500 non-refundable deposit payable by 29th October 2004, balance due by 30th January 2005
     ii. Late: ZAR 2 800: ZAR 500 non-refundable deposit payable by 31st December 2004, balance due by 30th January 2005
     iii. Onsite registration: ZAR 4 500

Please note: All deposits are strictly non-refundable.

- Additional Deposit: South African Rand 100.00
Please note, all guests will be required by the Eskom Convention Centre to pay a re-
fundable cash deposit of **ZAR 100.00** on arrival.

- **Registration**
  All registrations are being dealt with online at [www.wits.ac.za/leprosy](http://www.wits.ac.za/leprosy)
  For enquiries regarding administrative matters relating to the conference, please contact Peter Laubscher, Executive Director, Leprosy Mission Southern Africa at Email: peter@tlm.co.za, Phone: +27 11 440 6323, Fax: +27 11 440 6324, Mail: P O Box 46002 Orange Grove 2119 South Africa

- **Abstracts of papers**
  All scientific matters relating to the Congress are being co-ordinated by a scientific committee chaired by Dr. Noordeen, President of the ILA. Abstracts should be 200 words or less in length and should have a title and the names of the authors, including the name of the presenting author which should be underlined. The abstract should mention the affiliations of the authors and the address of the presenting author, including an email address. Delegates wishing to present papers should send their abstracts to Dr. Noordeen by 29th October: Email: Noordeen@eth.net, Phone: [91-44] 2445 6337, Fax: [91-44] 2445 6338, Mail: 1-A, K.G. Valencia 57, 1st Main Road, Gandhinagar, Adyar, Chennai – 600 020 INDIA.

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**National Conference on Elimination of Leprosy – Raipur (India).**

**Summary Report.**

Through an initiative taken by the International Leprosy Association, the National Conference on Elimination of Leprosy was organized under the auspices of Government of India and the Government of Chhattisgarh. The Conference was co-sponsored by the World Health Organization, the International Federation of Anti-leprosy Associations and the Nippon Foundation/the Sasakawa Memorial Health Foundation. The Conference was also supported by the International Leprosy Union, the Hind Kusht Nivaran Sangh, the Indian Association of Leprologists, IDEA and the Leprosy Elimination Alliance.

The Conference which started its deliberations on 27 January had its formal inaugural ceremony on the morning of the following day, 28 January, when it was formally inaugurated by His Excellency the President of India, Dr. A. P. J. Abdul Kalam, in the presence of H.E. the Governor of Chhattisgarh, Lt. Gen. K. M. Seth, the Chief Minister of Chhattisgarh Dr. Raman Singh, the Minister of Health Dr. Krishna Murthy Bandhi, Mr. Yohei Sasakawa, President of the Nippon Foundation, Dr. S. K. Noordeen, President of the International Leprosy Association and Chairman of the Organizing Committee, and Dr. C. S. Walter, Secretary of the International Leprosy Association and Secretary-General of the Organizing Committee.

The President of India, in his inaugural address, after greeting the participants, made a fervent plea to take concerted steps so that the prevalence of leprosy can be brought down to less than one case per 10,000 population by the year 2005. He emphasized the need for “rehabilitation with compassion” so that all treated patients are rehabilitated. In this connection, he commended the efforts made by Gujarat through a comprehensive program of leprosy elimination, disability care, and rehabilitation of leprosy patients in which the state government, medical colleges, hospitals and NGOs were active partners. He emphasized the need to study the increased occurrence of the disease in certain states and suggested introducing appropriate environmental remedial measures. He linked leprosy elimination to overall development at every level that would provide better amenities, hygiene, education, and better health care, and emphasized the vital need for a holistic approach. He concluded that leprosy elimination was a multi-dimensional task and asked the participants to work with the theme “Let my brain remove the pain of the suffering humanity.” His clarion call was concerted action by all so that the country is free from the disease within the prescribed time frame.

Dr. Raman Singh, the Chief Minister outlined the progress being made by Chhattisgarh state towards elimination of leprosy and promised that his state would reach the elimination target in time.

Mr. Yohei Sasakawa greeted the participants, and explained the Nippon Foundation’s commitment to the elimination of leprosy in India. He outlined its contribu-
tion through his trips to different states in India and the need to strengthen political commitment, leprosy awareness in the community and social support to leprosy affected persons.

Dr S. K. Noordeen welcomed the President of India and other dignitaries and outlined the objectives of the conference and how the conference was in tune with the ideals of Mahatma Gandhi and his objective of ending leprosy in the country. Dr. C. S. Walter proposed a vote of thanks.

The conference, which brought together over 900 participants from different parts of the country, had its sessions so structured to include invited lectures and organized symposia. There were five lectures on technical subjects covering case-detection and diagnosis (Dr. Krishnamurthy), MDT and management of leprosy in the field (Dr. Raja Rao), disability situation in India (Dr. Srinivasan), urban leprosy (Dr. Revankar), and community mobilization for leprosy elimination (Dr. Murugesan). There were eight symposia moderated by experts in their respective fields. These were operational and epidemiological issues in case-detection (Dr. Ravi Kumar), treatment delivery, case-holding and referral (Dr. Ranganatha Rao), rehabilitation within the community (Dr Gopal), reaching the unreached (Dr. Lobo), vision beyond 2005 (Dr Noordeen), integration of leprosy into general health services (Dr. Abraham Joseph), the role of media (Dr. Gokhale), and the role of NGOs in leprosy elimination (Dr. Walter). In addition to the lectures on technical topics, presentations were made on the progress towards leprosy elimination at national and state levels, including the constraints faced and remedial measures undertaken. The presentation on the national leprosy situation was made by Dr. Dhillon, Deputy Director-General of Health Services (Leprosy), Government of India. Presentations on the leprosy situation in the states of Andhra Pradesh, Chhattisgarh, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Orissa, and Tamil Nadu were made by the respective state leprosy officers. The sessions were well attended and there was considerable discussion by the participants on the various issues directly related to elimination of leprosy. Participants, who wished to present their own findings and views, particularly from the field, were able to do so through poster presentations and over 150 posters were presented.

The conference ended with a concluding session chaired by Dr. Krishna Morthy Bandhi, Minister of Health. The conclusions of the conference in the form of Raipur Declaration (see later) was read out by Dr Noordeen. Concluding remarks were made by Dr. Alok Shukla, Health Secretary, Dr. Ratre, Joint Director, Dr. Yuasa, Dr. Noordeen, Dr. Walter, and Dr. Masih.

**RAIPUR DECLARATION.** The National Conference on Elimination of Leprosy took place from 27 to 30 January 2004 at Raipur (Chhattisgarh) at the initiative of the International Leprosy Association. It was co-sponsored by World Health Organization (WHO), the International Federation of Anti-Leprosy Associations (ILEP) and The Nippon Foundation/Sasakawa Memorial Health Foundation. It was held under the auspices of the Government of India and the Government of Chhattisgarh and supported by the International Leprosy Union, the Hind Kusht Nivaran Sangh, the Indian Association of Leprologists, IDEA, and the Leprosy Elimination Alliance.

The National Conference after –

- Reviewing the current leprosy situation in the country and in the different states
- Appreciating the progress made in reducing the burden of leprosy in the country through widespread application of MDT along with related activities,
- Expressing concern over the high prevalence and case-detection in some of the states particularly the states of Bihar, Chhattisgarh, Jharkhand, Orissa, Uttar Pradesh, and West Bengal,
- Further expressing concern over the general tendency towards complacency as a result of initial successes,
- Recognizing the urgency of the situation in view of the approaching target date of December 2005,

Urges the national and state programs to:

1. **PROMOTE** more vigorously the integration of leprosy services into general health services so that service coverage is increased effectively and the goal of leprosy elimination reached in time;
2. **INTENSIFY VIGOROUSLY** their efforts towards detection of cases in time and completion of treatment;
3. **DISSEMINATE** appropriate IEC messages on signs and symptoms of leprosy, its curability through MDT, and the free availability of treatment in all health facilities so that patients can self-report in time;

4. **TAKE** steps to rehabilitate leprosy-affected persons in a cost-effective and compassionate manner so that they can be fully assimilated within the family and the community as productive members;

5. **ENSURE** that leprosy patients continue to get the necessary treatment services even after leprosy is eliminated as a public health problem;

6. **ADDRESS** the following specific problems or issues, the resolution of which is vital to the attaining of the goal of elimination of leprosy:

   - Continued problem of detecting all the backlog and hidden cases,
   - Delayed diagnosis,
   - Overdiagnosis,
   - Re-registration,
   - Inadequate and inappropriate training of general health services workers, hindering their effective participation in the integrated setting, and
   - Inability to dismantle unnecessary vertical elements in the programme.

7. **DEVELOP** strategies in collaboration with all stakeholders to identify and focus on specific geographic areas or populations of high endemicity and ensure that more intensified decentralized time bound plans are developed and implemented on an urgent basis, taking into consideration the priority problems mentioned above.
Leprosy remains an important health problem worldwide. The disease is caused by a chronic granulomatous infection of the skin and peripheral nerves with *Mycobacterium leprae*. The clinical range from tuberculoid to lepromatous leprosy is a result of variation in the cellular immune response to the mycobacterium. The resulting impairment of nerve function causes the disabilities associated with leprosy. This review summarises recent advances in understanding of the biology of leprosy, clinical features of the disease, the current diagnostic criteria, and the new approaches to treatment of the infection and the immune-mediated complications. Supervised multi-drug therapy (MDT) for fixed durations is highly effective for all forms of the disease. The widespread implementation of MDT has been associated with a fall in the prevalence of the leprosy but as yet no reduction in the case-detection rate globally. Thus, leprosy control activities must be maintained for decades to interrupt transmission of infection.—Authors’ Abstract


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

Decentralization policies are an integrated component of health sector reform in an increasing number of countries. The ability of such policies to improve the health system’s quality and efficiency is backed up by limited scientific evidence. This study intends to evaluate the impact of decentralization on a specialized field of disease control (leprosy control) in Colombia and Brazil. It analyses the respective juridical base, epidemiological indicators and local publications. Furthermore, 39 semi-structured interviews with key informants were conducted. In both countries, the devolution of technical responsibility and financial resources to the municipalities was the implemented form of decentralization. Access to preventive and curative health care and the community participation in decision-making improved clearly only in Brazil. The decentralization to private providers in Colombia had dubious effects on service quality in general and still more on public health. The flow of finances (including finance collection through state-owned taxes instead of insurance companies) seemed to be better controlled in Brazil. Leprosy control in Brazil took advantage of the decentralization process; in Colombia, it came close to a collapse.—Authors’ Abstract


This article discusses chapters 15 and 16 of the ancient midrash (allegorical commentary) Leviticus Rabba (IV–V AD) and its view of leprosy. The phenomenon of Biblical leprosy is here not investigated from a paleo-pathological point of view. The focus lies on its physiological, aetiological, pathological and therapeutic aspects as represented in Leviticus Rabba. It is argued that the medical views of Leviticus Rabba show a certain resemblance to some of the view of the Hippocratic School, notably with respect to humoral theory, the belief in the correspondence between the macrocosm and the individual microcosm, and the notion of paideia as a way of healing. Finally, it is shown that the ancient myth of the two floods (of water and of fire) is connected to the understanding of leprosy in Leviticus Rabba.—Author’s Abstract


Although the preventive action of dapsone against \textit{P. falciparum} malaria was known for many years, there was no report about the incidence of \textit{P. falciparum} malaria in leprosy patients treated with dapsone, especially from areas of Southeast Asia where both leprosy and malaria are endemic. Therefore, two clinic-based malaria surveys were undertaken at a gap of 12 years, comprising 506 lepromatous leprosy patients and 499 febrile nonleprosy control subjects. Both the surveys showed that the lepromatous patients treated with MDT had only \textit{P. vivax} malaria (incidence comparable to the febrile nonleprosy controls) with complete
freedom from *P. falciparum*. On the contrary, control subjects not taking any-leprosy drugs and staying with the leprosy patients at the same beggars’ home, had both *P. vivax* and *P. falciparum* malaria. It is postulated that dapsone provided protection against *P. falciparum* among leprosy patients.—Authors’ Abstract

**Smit, J. J., Folkerts, G., and Nijkamp, F.**


**PURPOSE OF REVIEW:** The “hygiene hypothesis” suggests that a relationship exists between improved hygiene and an increase in allergic diseases. As an underlying mechanism for this hypothesis it is proposed that due to the lack of microbial stimulation either a misbalance in T helper type responses or a misbalance in regulatory mechanisms develops. As yet, however, a specific infectious factor responsible for the hygiene hypothesis has not been found. **RECENT FINDINGS:** Animal models have lent support for mycobacteria as important candidates in the hygiene hypothesis. These animal studies have also suggested that mycobacterial treatment generated regulatory mechanisms which restored the immune balance. In contrast, the relationship between mycobacterial infection or treatment and the development of allergy and asthma in humans is unclear and highly controversial. **SUMMARY:** Mycobacteria have been found to unambiguously reduce allergic and asthmatic manifestations, suggesting that mycobacteria perhaps can be used as an “anti-asthma” vaccine. Conflicting results in humans, however, confirm that the complex and multifactorial interactions between the environment and the genetic background of the individual contribute to the development of allergic disease. Therefore, the hygiene hypothesis should involve the genetic and the environmental background of the individual.—Authors’ Abstract

**Verma, G., Upshur, R. E., Rea, E., and Benatar, S. R.**


**BACKGROUND:** Tuberculosis is a major cause of morbidity and mortality globally. Recent scholarly attention to public health ethics provides an opportunity to analyze several ethical issues raised by the global tuberculosis pandemic. **DISCUSSION:** Recently articulated frameworks for public health ethics emphasize the importance of effectiveness in the justification of public health action. This paper critically reviews the relationship between these frameworks and the published evidence of effectiveness of tuberculosis interventions, with a specific focus on the controversies engendered by the endorsement of programs of service delivery that emphasize direct observation of therapy. The role of global economic inequities in perpetuating the tuberculosis pandemic is also discussed. **SUMMARY:** Tuberculosis is a complex but well understood disease that raises important ethical challenges for emerging frameworks in public health ethics. The exact role of effectiveness as a criterion for judging the ethics of interventions needs greater discussion and analysis. Emerging frameworks are silent about the economic conditions contributing to the global burden of illness associated with tuberculosis and this requires remediation—Authors’ Abstract


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

**Chemotherapy**


Descriptors for thalidomide and its N-alkyl derivatives have been obtained from solubilities reported by Goosen, et al. It is shown that thalidomide and the three N-alkylthalidomides are reasonably strong hydrogen bond bases, and that thalidomide is a hydrogen bond acid. From the obtained descriptors, a large number of physicochemical and biochemical properties of the four thalidomides were predicted, including several water-solvent partition coefficients. Predictions of skin permeation of the four thalidomides are in excellent agreement with experiment. Thalidomide is predicted to be only moderately to poorly distributed to the brain, but N-pentylthalidomide, is predicted to be very well distributed to the brain. All four thalidomides have very high predicted % human intestinal absorption.—Author’s Abstract


Extracts obtained from three Nigerian Sterculiaceae plants: Cola acuminata, C. nitida and C. milleni were screened for antimycobacterium properties using a slow growing Mycobacterium bovis ATCC 35738 (designated BCG Mexican and known to have some virulence in mouse and guinea pig) at 1000 microg/ml using the radiometric (BACTEC) method. The extracts were also tested against six fast growing ATCC strains of M. vaccae using the broth microdilution method. The methanol extracts from both leaves, stem bark and root bark of Cola acuminata and from the leaves and stem bark of C. nitida and C. milleni were not active at the highest concentration of 1000 microg/ml. Only the methanol extract of root bark for both C. nitida and C. milleni were found to be potent against both M. bovis and strains of M. vaccae. The minimum inhibitory concentration (MIC) of C. nitida against M. bovis is 125 microg/ml while the MIC of C. milleni against M. bovis is 62.5 microg/ml after at least 6 days of inhibition with growth index (GI) units lesser than or equal to the change in GI units inoculated with a 1/100 of the BACTEC inoculum for a control vial. The minimum inhibitory concentration of C. milleni against the six ATCC strain of M. vaccae ranged from 62.5 microg/ml to 250 microg/ml while for C. nitida ranged from 500 microg/ml to above 1000 microg/ml. Evidently, C. milleni has the highest inhibitory activity against both M. bovis and strains of M. vaccae used. Rifampicin, the positive control used has strong activity against M. bovis at the tested concentration of 5 microg and 10 microg/ml and 4 to 8 microg/ml against the six strains of M. vaccae.—Authors’ Abstract


OBJECTIVE: To investigate if rifampicin is both an inducer and an inhibitor of repaglinide metabolism, it was determined whether the timing of rifampicin co-
administration influences the pharmacokinetics of repaglinide. METHODS: Male volunteers (N = 12) participated in a randomised, two-period, crossover trial evaluating the effect of multiple doses of 600 mg rifampicin once daily for 7 days on repaglinide metabolism. Subjects were, after baseline measurements of repaglinide pharmacokinetics, randomized to receive, on either day 7 or day 8 of the rifampicin administration period, a single dose of 4 mg repaglinide and vice versa in the following period. RESULTS: When repaglinide was given, together with the last rifampicin dose, on day 7, an almost 50% reduction of the median repaglinide area under the plasma concentration-time curve (AUC) was observed. Neither the peak plasma concentration (C(max)), time to reach C(max) (t(max)) nor terminal half-life (t(1/2)) was statistically significantly affected. When repaglinide was given on day 8, 24 hr after the last rifampicin dose, an almost 80% reduction of the median repaglinide AUC was observed. The median C(max) was now statistically significantly reduced from 35 ng/ml to 7.5 ng/ml. Neither t(max) nor t(1/2) was significantly affected. CONCLUSION: When rifampicin and repaglinide are administered concomitantly, rifampicin seems to act as both an inducer and an inhibitor of the metabolism of repaglinide. After discontinuing rifampicin administration, while the inductive effect on CYP3A4 and probably also CYP2C8 is still present, an even more marked reduction in the plasma concentration of repaglinide was observed. Our results suggest that concomitant administration of rifampicin and repaglinide may cause a clinically relevant decrease in the glucose-lowering effect of repaglinide, in particular when rifampicin treatment is discontinued or if the drugs are not administered simultaneously or within a few hours of each other.—Authors’ Abstract


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


We have used a phospholipase C (PLC)-deletion mutant (plcABC) of the H37Rv strain of Mycobacterium tuberculosis (MTB), as well as a plcA-insertion mutant of Mycobacterium smegmatis, to investigate the possible involvement of PLCs in clofazimine-mediated inhibition of mycobacterial K(+) transport and growth. Inactivation of the PLCs of MTB and insertion of the plcA gene into M. smegmatis resulted in a substantial reduction and increase in hydrolysis of phosphatidylcholine (PC), respectively. However, both the mutant and wild-type strains of MTB and M. smegmatis were equally sensitive to the inhibitory effects of clofazimine on K(+) uptake and growth. These observations demonstrate that the PLCs of MTB are not involved in the antimicrobial activity of clofazimine.—Authors’ Abstract

Despite its history as a human teratogen, thalidomide is emerging as a treatment for cancer and inflammatory diseases. Although the evolution of its clinical application could not have been predicted from the tragedy associated with its misuse in the past, its history serves as a lesson in drug development that underscores the need to understand the molecular pharmacology of a compound’s activity, including associated toxicities. Here, we summarise the applications for thalidomide with an emphasis on clinical trials published over the past 10 years, and consider our knowledge of the molecular pharmacology of the drug in the context of clinical trial data, attempting to provide a mechanism-guided understanding of its activity.—Authors’ Abstract


The potential of ligand binding proteins as drug carriers and delivery systems has recently sparked great interest. We investigated the potential of tear lipocalin (TL) to bind the antibiotic, rifampin, and the environmental conditions for controlled release. To determine if TL binds rifampin, gel filtration was used to isolate protein fractions of tears. Rifampin was detected by absorbance spectroscopy in the elution fractions containing TL. The bound complex of rifampin-TL generates optical activity at about 360 nm, indicating a unique conformation at the binding site. Rifampin has a higher affinity for TL (Kd = 128 microM) than albumin. Rifampin is released from the TL calyx in acidic conditions and is displaced by palmitic acid. Autooxidation of free rifampin begins in minutes but is delayed by at least 3 hr in the presence of TL. These properties are conducive to stabilization and delivery of rifampin to tubercles that are acidic and rich in fatty acids. These studies show the potential of TL as a carrier for rifampin with controlled release to a targeted environment.—Authors’ Abstract


Ofloxacin(OFLX) is often applied today as a substitution drug of MDT for drug resistance to dapsone, rifampicin or clofazimine. However, OFLX resistance is also becoming a great concern. Low and/or irregular administration are considered to be the major causes of OFLX resistance. OFLX should be used as a combined therapy, and minimal daily dose of 400 mg of OFLX or 200–300 mg of levofloxacin is required. Quinolone resistance should be considered when no improvement of clinical and/or bacterial index is observed after the treatment for 6 months. In such cases, resistance gene detection is necessary.—Authors’ Abstract


Taking into consideration the benefits of the combined therapy of isoniazid (INH) and rifampicin (RIF), this study focused on co-encapsulation of INH and RIF in the same liposome formulation. INH was incorporated in the aqueous phase and RIF in the lipid layer. Liposomes containing either INH or RIF were also prepared. All liposome formulations were compared for their loading capacity, encapsulation percentage and release properties. Drug amounts in the liposomes were estimated using peak-to-peak first-order derivative UV spectroscopy. Among the liposome formulations DPPC:chol liposomes showed the highest loading capacity (106.70 ± 0.12 for INH and 18.17 ± 0.06 (×10(–3)) for RIF) and encapsulation percentage (73.84 ± 0.78 for INH and 81.53 ± 2.06 for RIF) compared to EPC:chol liposomes.
(loading capacity 93.36 ± 0.58 for INH and 17.87 ± 0.11 (×10−3) for RIF; encapsulation percentage 64.61 ± 0.51 for INH and 74.45 ± 0.48 for RIF). Co-encapsulation of INH and RIF increased their individual encapsulation percentage and extended drug release compared to the formulations containing drug alone (Table 2). Results of this study support the conclusion that lipid and water soluble drugs can be successfully co-encapsulated in the same liposome formulation and also show that derivative UV spectroscopy is a sensitive method for direct and accurate quantification of these co-encapsulated drugs.—Authors’ Abstract


Studies have shown that CYP2C9.1 mediated metabolism of flurbiprofen or naproxen is activated by co-incubation with dapsone. However, dapsone activation has not been examined in the known variant forms of CYP2C9. Six concentrations of flurbiprofen (2–300 microM) or naproxen (10–1800 microM) were co-incubated with six concentrations of dapsone (0–100 microM) and with reconstituted, purified CYP2C9.1, CYP2C9.2 (R144C), CYP2C9.3 (I359L), or CYP2C9.5 (D360E), in order to assess degrees of activation. Dapsone increased the efficiency (V(m)/K(m)) of flurbiprofen 4′-hydroxylation by CYP2C9.1, CYP2C9.2, CYP2C9.3, and CYP2C9.5 by 8-, 31-, 47-, and 22-fold, respectively. In similar experiments using the substrate naproxen, dapsone increased the efficiency of naproxen demethylation 7-, 15-, 13-, and 22-fold, in CYP2C9.1, CYP2C9.2, CYP2C9.3, and CYP2C9.5, respectively. Also, dapsone normalized naproxen’s kinetic profile from biphasic (CYP2C9.1 and CYP2C9.2) or linear (CYP2C9.3 and CYP2C9.5) to hyperbolic for all variant forms. Thus, amino acid substitutions of CYP2C9 variants affect the degree of dapsone activation in a genotype-dependent fashion. Furthermore, the degree of effect noted across variants appeared to be dependent on the substrate studied.—Authors’ Abstract


The Mycobacterium tuberculosis rmlC gene encodes dTDP-4-keto-6-deoxyglucose epimerase, the third enzyme in the M. tuberculosis dTDP-L-rhamnose pathway which is essential for mycobacterial cell-wall synthesis. Because it is structurally unique, highly substrate-specific and does not require a cofactor, RmlC is considered to be the most promising drug target in the pathway, and the M. tuberculosis rmlC gene was selected in the initial round of TB Structural Genomics Consortium targets for structure determination. The 1.7 A native structure determined by the consortium facilities is reported and implications for in silico screening of ligands for structure-guided drug design are discussed.—Authors’ Abstract


Thalidomide, 2-(2,6-dioxo-3-piperidinyl)-1H-isindole-1,3(2H)-dione, has been shown to inhibit angiogenesis, the formation of new blood vessels from existing vasculature. As a result, there is renewed interest in this drug as a potential therapy for solid tumors. Thalidomide forms a number of metabolites and has been shown to require metabolic activation for antiangiogenic activity. A series of 39 compounds, based upon the structure of some of these metabolites, was synthesized and tested for their ability to inhibit microvessel growth in the rat aortic ring assay. The results of this testing have been used as the basis for a three-dimensional quantitative structure-activity relationship (3D-QSAR) study, utilizing comparative
molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) procedures. The best resulting CoMFA and CoMSIA models have conventional r(2) values of 0.924 and 0.996, respectively. The cross-validated q(2) values are 0.666 and 0.635, respectively. These models offer insight into the structural requirements for activity of thalidomide analogues as angiogenesis inhibitors, since there is only speculative knowledge of the target. Additionally, it appears as though there is more than one active site or mechanism of action.—Authors’ Abstract


The therapy of mycobacterial infections is challenging for a number of reasons. Because mycobacteria are not susceptible to many classes of antibacterial agents, treatment typically requires the use of antimicrobial drugs that are not commonly used and may have small therapeutic windows. For many species, procedures for drug susceptibility testing and optimal treatment regimens have yet to be defined. Finally, because mycobacteria are generally slow to succumb to antimicrobial agents, therapy must be given with multiple drugs for prolonged periods of time, making it necessary to monitor for drug toxicity, drug interactions, and patient nonadherence. Better understanding of the pharmacokinetics and pharmacodynamics of antimycobacterial agents should improve the therapy of mycobacterial infections. Using current treatment strategies for tuberculosis and Mycobacterium avium complex infections as examples, this review highlights basic pharmacokinetic and pharmacodynamic principles and the rationale for combination chemotherapy that should also be applicable to other mycobacterial infections.—Authors’ Abstract


OBJECTIVES: This study was designed to develop alginate-chitosan microspheres as drug carriers to reduce dose/dosing frequency in the management of tuberculosis (TB), which otherwise demands prolonged chemotherapy. METHODS: Alginate-chitosan microspheres encapsulating three frontline anti-tuberculous drugs (ATDs), rifampicin, isoniazid and pyrazinamide, were formulated. A therapeutic dose and a half-therapeutic dose of the microsphere-encapsulated ATDs were orally administered to guinea pigs for pharmacokinetic/chemotherapeutic evaluations, respectively. RESULTS: The drug encapsulation efficiency ranged from 65% to 85% with a loading of 220–280 mg of drug per gram microspheres. Administration of a single oral dose of the microspheres to guinea pigs resulted in sustained drug levels in the plasma for 7 days and in the organs for 9 days. The half-life and mean residence time of the drugs were increased 13- to 15-fold by microsphere encapsulation, along with an enhanced relative/absolute bioavailability. The sustained release and increase in bioavailability were also observed with a sub-therapeutic dose of the microspheres. In Mycobacterium tuberculosis H37Rv-infected guinea pigs, administration of a therapeutic dose of microspheres spaced 10 days apart produced a clearance of bacilli equivalent to conventional treatment for 6 weeks. The most important observation, however, was the documentation of therapeutic benefit with a half-therapeutic dose of the microspheres administered weekly. CONCLUSION: Alginate-chitosan microspheres hold promise as a potential natural polymer-based oral ATD carrier for better management of TB.—Authors’ Abstract


Aminoglycoside use is limited by ototoxicity and nephrotoxicity. This study compared the incidences of toxicities associated
with 2 recommended dosing regimens. Eighty-seven patients with tuberculosis or nontuberculous mycobacterial infections were prospectively randomized by drug to receive 15 mg/kg per day or 25 mg/kg 3 times per week of intravenous streptomycin, kanamycin, or amikacin. Doses were adjusted to achieve target serum concentrations. The size of the dosage and the frequency of administration were not associated with the incidences of ototoxicity (hearing loss determined by audiogram), vestibular toxicity (determined by the findings of a physical examination), or nephrotoxicity (determined by elevated serum creatinine levels). Risk of ototoxicity (found in 32 [37%] of the patients) was associated with older age and with a larger cumulative dose received. Vestibular toxicity (found in 8 [9%] of the patients) usually resolved, and nephrotoxicity (found in 13 [15%] of the patients) was mild and reversible in all cases. Subjective changes in hearing or balance did not correlate with objective findings. Streptomycin, kanamycin, and amikacin can be administered either daily or 3 times weekly without affecting the likelihood of toxicity.—Authors' Abstract


OBJECTIVE: To evaluate the efficacy of erythrocytes loaded with the haemolytic toxin listeriolysin O against Mycobacterium avium replication within macrophages. METHODS: Recombinant listeriolysin O was loaded in human erythrocytes by a procedure of hypotonic dialysis and isotonic resealing. Loaded erythrocytes were modified to allow them to be recognized and taken up by human macrophages infected with M. avium. The antimycobacterial activity of the erythrocytes loaded with listeriolysin O was evaluated by supernatant and intracellular CFU counts on days 4 and 7 post-erythrocyte administration. RESULTS: Recombinant listeriolysin O was encapsulated in human erythrocytes to reach final concentrations ranging from 1 to 4 ng/mL of erythrocytes. Erythrocytes loaded with increasing quantities of recombinant protein were able to reduce (at most by 50%) M. avium replication in a dose-dependent fashion when administered to infected macrophages. CONCLUSIONS: Erythrocytes loaded with listeriolysin O are effective against M. avium (such as Mycobacterium tuberculosis and Mycobacterium leprae) by itself or as part of an antimycobacterial treatment.—Authors' Abstract


Thalidomide is a racemic glutamic acid derivative approved in the US for erythema nodosum leprosum, a complication of leprosy. In addition, its use in various inflammatory and oncologic conditions is being investigated. Thalidomide interconverts between the (R)- and (S)-enantiomers in plasma, with protein binding of 55% and 65%, respectively. More than 90% of the absorbed drug is excreted in the urine and faeces within 48 hr. Thalidomide is minimally metabolised by the liver, but is spontaneously hydrolysed into numerous renally excreted products. After a single oral dose of thalidomide 200 mg (as the US-approved capsule formulation) in healthy volunteers, absorption is slow and extensive, resulting in a peak concentration (C(max)) of 1–2 mg/L at 3–4 hours after administration, absorption lag time of 30 minutes, total exposure (AUC (infinity)) of 18 mg hr/L, apparent elimination half-life of 6 hours and apparent systemic clearance of 10 L/hr. Thalidomide pharmacokinetics are best described by a one-compartment model with first-order absorption and elimination. Because of the low solubility of the drug in the gastrointestinal tract, thalidomide exhibits absorption rate-limited pharmacokinetics (the ‘flip-flop’ phenomenon), with its elim-
ination rate being faster than its absorption rate. The apparent elimination half-life of 6 hours therefore represents absorption, not elimination. The ‘true’ apparent volume of distribution was estimated to be 16L by use of the faster elimination-rate half-life. Multiple doses of thalidomide 200 mg/day over 21 days cause no change in the pharmacokinetics, with a steady-state C(max) (C(ss)(max)) of 1.2 mg/L. Simulation of 400 and 800 mg/day also shows no accumulation, with C(ss)(max) of 3.5 and 6.0 mg/L, respectively. Multiple-dose studies in cancer patients show pharmacokinetics comparable with those in healthy populations at similar dosages. Thalidomide exhibits a dose-proportional increase in AUC at doses from 50 to 400 mg. Because of the low solubility of thalidomide, C(max) is less than proportional to dose, and t(max) is prolonged with increasing dose. Age, sex and smoking have no effect on the pharmacokinetics of thalidomide, and the effect of food is minimal. Thalidomide does not alter the pharmacokinetics of oral contraceptives and is also unlikely to interact with warfarin and grapefruit juice. Since thalidomide is mainly hydrolysed and passively excreted, its pharmacokinetics are not expected to change in patients with impaired liver or kidney function.—Authors’ Abstract


Clarithromycin (CAM) and rifampicin (RFP) have both been recognized to be effective antibiotic agents against Mycobacterium avium complex (MAC) infection. Rifamycin derivatives including RFP and rifabutin modulate the CAM metabolism by inducing the hepatic cytochrome p-450 3A4. To clarify the effect of RFP on the CAM metabolism, we measured the plasma concentration of CAM and 14-R-hydroxyclarithromycin (M-5), the major metabolite of CAM, in 9 patients suffering from MAC infection before and after the addition of RFP. After the addition of RFP, the mean plasma concentration of CAM significantly decreased, while that of M-5 did not. In addition, the amount of CAM + M-5 concentration also significantly decreased. As M-5 is less effective against MAC infection than CAM, more attention should thus be paid to the plasma CAM concentration in patients administered CAM and RFP concomitantly.—Authors’ Abstract


Monthly doses of rifampin, ofloxacin, and minocycline (ROM) are expected to be effective treatment for multi-bacillary leprosy. Patients with MB leprosy received ROM (N = 10) or World Health Organization multi-drug therapy (MDT) (N = 11). Treatment with ROM was given as 24 consecutive monthly observed doses of rifampin (600 mg), ofloxacin (400 mg), and minocycline (100 mg). Treatment with MDT was given as 24 consecutive monthly observed doses of rifampin (600 mg) and clofazimine (300 mg), and unobserved daily dapsone (100 mg) and clofazimine (50 mg). Twenty patients completed the 24-month regimens with >99% compliance. Treatments with ROM and MDT were safe, tolerable, and caused similar improvements in lesions, bacterial indices, and histology. All MDT recipients developed clofazimine-induced pigmentation. Six ROM and nine MDT recipients assessed at five or more years after completion of treatment had no evidence of relapse. Twenty-four months of treatment with ROM is a safe, well-tolerated, and convenient regimen that may provide an alternate therapy to MDT for MB leprosy. Larger trials with sufficient follow-up would better define the role of ROM.—Authors’ Abstract
Histoid leprosy is a particular variant of lepromatous leprosy presenting as cutaneous or subcutaneous nodular and/or plaque-like lesions arising from apparently normal skin. It is characterized histologically by spindle-shaped histiocytes in interlacing bundles and whorls, containing numerous intact and rod-shaped *Mycobacterium leprae*. It can occur de novo or secondary in patients treated for a long course by dapsone alone. We describe a case of lepromatous leprosy treated according to the national Moroccan protocol who developed histoid lesions during his treatment by dapsone. The patient responded well to fluoroquinolone, rifampicin and clofazimine, with however, the occurrence of erythema nodosum leprosum.—Authors’ Abstract

Melkersson-Rosenthal (MRS) syndrome is characterized by a classical triad of recurrent or persistent orofacial swelling, peripheral facial nerve paralysis and lingua plicata. Granulomatous cheilitis (GC) is regarded as a monosymptomatic form of MRS. The exact aetiologies of MRS and GC are unknown. In this study we investigated the possible role of mycobacteria in these two conditions. A ribosomal RNA amplification-based Gen-Probe amplified *Mycobacterium tuberculosis* direct test was used to investigate the presence of *M. tuberculosis* complex in paraffin-embedded skin biopsy specimens from five patients with MRS and one patient with GC. Three of the six specimens were shown to be positive using this system; one of the positive specimens also showed positive Ziehl-Neelsen staining. These results suggest a possible mycobacterial aetiology for MRS and GC.—Authors’ Abstract

PURPOSE: To determine the association of demographics, leprosy and ocular characteristics with altered levels of lactoferrin in the tears of normal subjects and leprosy patients, and to detect the presence of antibodies to lactoferrin in these tear samples. METHOD: We collected light-stimulated tears from 298 leprosy patients and an equal number of normal subjects using the glass capillary method. Free lactoferrin levels were estimated using ELISA and the presence of antibodies to lactoferrin was detected using the immuno-blotting method. Significant associations were looked for between tear lactoferrin levels and demographic characteristics, leprosy characteristics such as type of disease, duration of disease, reactions, deformity and bacterial load, and ocular complications, using chi-square and regression analysis. RESULTS: Tear lactoferrin levels with a mean (S.D.) of 2.55 (2.83) mg/ml in the control group were significantly different (p <0.000) from leprosy patients with a mean (S.D.) of 5.66 (7.21)mg/ml. Age showed an inverse correlation with tear lactoferrin levels in controls. Increased bacterial load, grade 2 leg deformity and Type 2 reactions were significantly associated (p <0.05) with increased tear lactoferrin levels. Type 2 reactions remained significantly associated (p = 0.01) on multiple regression analysis. Tear lactoferrin levels were not associated with gender, serum lactoferrin levels, Type 1 reactions, face patches, treatment status, orbicularis ocularis weakness, lagophthalmos, entropion, entropion, corneal opacity, cataract and iridocyclitis. CONCLUSION: Age is inversely related to tear lactoferrin levels in normal subjects. Free lactoferrin levels in tears are
significantly higher in leprosy patients compared with normal controls. Type 2 reactions in leprosy are significantly associated with elevated tear lactoferrin levels.—Authors’ Abstract


We report a patient with lepromatous leprosy who developed a rare variant of type-2 lepra reaction, characterized by pustular lesions, on switching from WHO multi drug therapy (MDT) to ofloxacin-aided MDT.—Authors’ Abstract


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


Tattoo inoculation borderline tuberculoid (BT) leprosy in upgrading reaction with prominent tattoo oedema developing after starting paucibacillary multidrug therapy (PB MDT) is reported. The diagnosis was confirmed by histopathology. An excellent response to oral steroids and PB MDT was seen. There is only one similar report in the literature.—Author’s Abstract


Chronic neuropathic pain in treated leprosy has received scant attention. In this article the concept, clinical features and diagnosis of neuropathic pain are reviewed. The
possible pathophysiological mechanisms, treatment challenges and research needs in this area are discussed.—Authors’ Abstract


Leprosy causes several ocular disorders, and it also causes aftereffect with high frequency in various ways. Primary impairment is the ocular disturbance caused with direct invasion of nerve and ocular tissue by Mycobacterium leprae. Secondary impairment is the complication of nerve paralysis and residual inflammation due to primary disorder. Main work at Japanese national leprosariums has been the control of primary and secondary impairment in recent years. Clinical ophthalmic study in the leprosarium revealed a increase of age-related ocular disease in addition to aftereffect of leprosy. Severe sequelae due to sensory and functional disturbance will require suitable applications of advanced clinical technologies.—Author’s Abstract


The bacteriological index (BI) of the skin smears is traditionally one of the important parameters of assessment of severity and of progress of leprosy under multidrug therapy. The present study reports on BI clearance among 578 multibacillary treated leprosy patients and the factors that influence this clearance. The patients were treated till smear negativity or for 2 years fixed duration and their skin smears periodically examined every 6 to 12 months till negativity (and even afterwards). We confirm that bacterial clearance is a slow process. The time taken for each log-unit decline in BI is between 13.6 to 24 months probably depending on initial BI level. The rate of smear negativity appears to be dependent on immune competence of the patients as reflected by a rapid BI decline in borderline BT-BB patients vis-a-vis BL-LL lepromatous patients both in the low and high BI group. Patients who had several episodes of ENL, took significantly longer time (63.7 months versus 53.5 months, p <0.0001) to become smear negative than those without ENL.—Authors’ Abstract


PURPOSE: Detailed ophthalmic evaluation was performed to determine the prevalence of ocular complications among leprosy patients on multidrug therapy and those released from multidrug treatment. DESIGN: Observational case series. METHODS: Leprosy patients at Tribhuvan University Teaching Hospital from April 1, 2001, through September 30, 2002, underwent detailed ophthalmic evaluation including slit-lamp biomicroscopy, dilated fundoscopy, and applanation tonometry. RESULTS: We evaluated 58 leprosy patients. A majority (72%) was receiving treatment for multibacillary leprosy; 14% belonged to posttreatment multibacillary and paucibacillary groups. Ocular involvement was found in 57% of patients. In the multibacillary group, 55% had ocular involvement, which was more than double that found in the paucibacillary group (25%), although this finding was not statistically significant (p = 0.187). Among patients with ocular complications, 48% had visual disability and another 45% had threatened vision; 9% met World Health Organization guidelines for blindness. Uveitis and its complications were the predominant causes of visual disability (88%). CONCLUSION: Ocular complications and visual disability are high among leprosy patients in Nepal even after completing multidrug therapy.—Authors’ Abstract


OBJECTIVE: To evaluate the presence of oral disease, as assessed by dental and periodontal indices, in the anterior maxilla of a group of 76 patients with leprosy, compared with a group of matched control subjects. MATERIALS AND METHODS: The study included 76 patients with leprosy (age range
40–82 years; 39 males), resident in the sanatorium of San Francisco de Borja de Fontilles (Alicante, Spain). Clinical examination was carried out to evaluate the decayed missing and filled index, and the periodontal status in the anterior maxilla, using the Loe-Silness dental plaque index, mean periodontal probing depth and the average periodontal attachment loss. RESULTS: In the leprosy patients, a large proportion of maxillary incisors and canines were missing. The mean plaque index in leprosy was 2.35 ± 0.7, with a probing depth of 2.96 ± 0.8, and an average attachment loss of 4.18 ± 1.3, indices all statistically greater than in controls. There were no differences detected in the oral indices measured according to the presence or absence of facial destruction or the type of leprosy. CONCLUSIONS: Patients with leprosy show a tendency to poor dental and periodontal health, unrelated to the presence of facial destruction or the type of leprosy.—Authors’ Abstract


Reported here are the cases of two HIV-positive patients with skin lesions suggestive of leprosy, based on clinical and pathological analysis, which worsened during the few weeks following initiation of highly active antiretroviral therapy. The lesions improved after a few weeks of multidrug therapy for leprosy. Mycobacterium leprae was confirmed by polymerase chain reaction analysis of blood in case 1 and of a biopsy sample in case 2. Neither Mycobacterium avium complex nucleic acid, which is usually associated with immune restoration syndrome, nor mycobacterial cutaneous manifestations were detected in either case.—Authors’ Abstract


BACKGROUND: Nerve function impairment (NFI) is the key outcome of the pathological processes of infection with Mycobacterium leprae, which can continue after completion of multidrug therapy (MDT) and lead to disability after leprosy patients are released from treatment. The objective of this study was to assess the need for and duration of surveillance of NFI. METHODS: Prospective cohort study of 2664 new leprosy patients in Bangladesh, with an observation period of 36 months in paucibacillary (PB) patients, and 60 months in multibacillary (MB) patients. Incidence rates (IR) were calculated with the number of patients developing NFI, type 1 and type 2 reactions, and silent neuritis for the first time after registration as the numerator, and cumulative person-years at risk (PYAR) as the denominator. Survival curves to the first event of NFI were also calculated. RESULTS: The IR of first event of NFI amongst MB patients was 16.1 per 100 PYAR, with 121/357 (34%) developing NFI during the observation period. Of the 121 with a first event of NFI, 77 (64%) had this within a year after registration, 35 (29%) in the second year, and the remaining 9 (7%) after 2 years. The IR of first event of NFI amongst PB patients was 0.9 per 100 PYAR, with 54/2153 (2.5%) developing NFI during the observation period. Of the 54 with a first event of NFI, 48 (89%) had this within a year after registration, 3 (5.5%) in the second year, and the remaining 3 (5.5%) cases after 2 years. The percentage of PB patients with no NFI at registration surviving without developing NFI during the observation period was 99% and for PB patients with NFI at registration 92%. In MB patients without NFI at registration, the percentage surviving with no NFI during the observation period was 84% and for MB patients with NFI at registration only 36%. CONCLUSION: New episodes of NFI and reactions after registration are common, in particular in MB patients with long-standing NFI at registration. The study highlights the importance of continuing surveillance for NFI of this risk group after registration for 2 years. Active surveillance beyond 2 years is not indicated.—Authors’ Abstract

DAP12 and its associating molecules MDL-1, TREM-1, and TREM-2 are the recently identified immune regulatory molecules, expressed primarily on myeloid cells including monocytes/macrophages, dendritic cells, NK cells, and neutrophils. However, little is known about the regulation of their expression during host antimicrobial responses. We have investigated the effect of pulmonary mycobacterial infection and type 1 cytokines on the expression of these molecules both in vivo and in vitro. While DAP12 was constitutively expressed at high levels in the lungs, the MDL-1, TREM-1, and TREM-2 molecules were inducible during mycobacterial infection. Their kinetic expression was correlated with that of the type 1 cytokines tumor necrosis factor alpha (TNF-alpha) and gamma interferon (IFN-gamma). In primary lung macrophage cultures, high constitutive levels of DAP12 and TREM-2 were not modulated by mycobacterial or type 1 cytokine exposure. In contrast, expression of both MDL-1 and TREM-1 was markedly induced by mycobacterial infection and such induction was inhibited by concurrent exposure to IFN-gamma. On mycobacterial infection of TNF-alpha(-/-) and IFN-gamma(-/-) mice in vivo or their lung macrophages in vitro, TNF-alpha was found to be critical for mycobacterially induced MDL-1, but not TREM-1, expression whereas IFN-gamma negatively regulated mycobacterially induced MDL-1 and TREM-1 expression. Our findings thus suggest that DAP12 and its associating molecules are differentially regulated by mycobacterial infection and type 1 cytokines and that MDL-1- and TREM-1-triggered DAP12 signaling may play an important role in antimicrobial type 1 immunity.—Authors’ Abstract


Total chemical protein synthesis was used to generate multimilligram quantities of the mechanosensitive channel of large conductance from *Escherichia coli* (Ec-MscL) and *Mycobacterium tuberculosis* (Tb-MscL). Cysteine residues introduced to allow chemical ligation were masked with cysteine-reactive molecules, resulting in side chain functional groups similar to those of the wild-type protein. Synthetic channel proteins were transferred to 2,2,2-trifluoroethanol and reconstituted into vesicle membranes. Fluorescent imaging of vesicles showed that channel proteins were membrane-localized. Single-channel recordings showed that reconstituted synthetic Ec-MscL has conductance, pressure dependence, and substate distribution similar to those of the recombinant channel. Reconstituted synthetic Tb-MscL also displayed conductance and pressure dependence similar to that of the recombinant protein. Possibilities for the incorporation of unnatural amino acids and biophysical probes, and applications of such synthetic ion channel analogs, are discussed.—Authors’ Abstract


The protective efficacy of *Mycobacterium bovis* BCG can be markedly augmented by stable integration of *Mycobacterium tuberculosis* genomic region RD1. BCG comple-
mented with RD1 (BCG::RD1) encodes nine additional proteins. Among them, 10-kDa culture filtrate protein (CFP-10) and ESAT-6 (6-kDa early secreted antigenic target) are low-molecular-weight proteins that induce potent Th1 responses. Using pools of synthetic peptides, we have examined the potential immunogenicity of four other RD1 products (PE35, PPE68, Rv3878, and Rv3879c). PPE68, the protein encoded by \( \text{rv}3873 \), was the only one to elicit gamma interferon (IFN-gamma)-producing cells in C57BL/6 mice infected with \( M. \) tuberculosis. Anti-PPE68 T cells were predominantly raised against an epitope mapped in the N-terminal end of the protein. Importantly, inactivation of \( \text{rv}3873 \) in BCG::RD1 did not modify CFP-10 and ESAT-6 secretion. Moreover, the generation of IFN-gamma responses to these antigens following immunization with BCG::RD1 was independent of PPE68 expression. Taken together, these results show that PPE68 is an immunogenic product of the RD1 region, which does not interfere with the secretion and immunogenicity of CFP-10 and ESAT-6.—Authors’ Abstract


The kinetics of activation and induction of several effector functions of human natural killer (NK) cells in response to \( M. \) bovis bacille Calmette-Guerin (BCG) were investigated. Owing to the central role of monocytes/macrophages (MM) in the initiation and maintenance of the immune response to pathogens, two different experimental culture conditions were analysed. In the first, monocyte-depleted nylon wool non-adherent (NW) cells from healthy donors were stimulated with autologous MM preinfected with BCG (intracellular BCG). In the second, the NW cells were directly incubated with BCG, which was therefore extracellular. In the presence of MM, CD4+ T lymphocytes were the cell subset mainly expressing the activation marker, CD25, and proliferating with a peak after 7 days of culture. In contrast, in response to extracellular BCG, the peak of the proliferative response was observed after 6 days of stimulation, and CD56+ CD3– cells (NK cells) were the cell subset preferentially involved. Such proliferation of NK cells did not require a prior sensitization to mycobacterial antigens, and appeared to be dependent upon contact between cell populations and bacteria. Following stimulation with extracellular BCG, the majority of interferon-gamma (IFN-gamma)-producing cells in C57BL/6 mice infected with \( M. \) tuberculosis. Anti-PPE68 T cells were predominantly raised against an epitope mapped in the N-terminal end of the protein. Importantly, inactivation of \( \text{rv}3873 \) in BCG::RD1 did not modify CFP-10 and ESAT-6 secretion. Moreover, the generation of IFN-gamma responses to these antigens following immunization with BCG::RD1 was independent of PPE68 expression. Taken together, these results show that PPE68 is an immunogenic product of the RD1 region, which does not interfere with the secretion and immunogenicity of CFP-10 and ESAT-6.—Authors’ Abstract


Macrophage apoptosis occurs within the granuloma, which is essential for successful immunity to tuberculosis. \textit{In vitro} macrophage apoptosis is associated with the killing of intracellular \( \text{Mycobacterium tuberculosis} \). A greater understanding of these observations will lead to new immunotherapies and improved vaccine design. The relevant apoptotic stimuli, the anti-mycobacterial mechanisms that they stimulate and their physiological relevance are reviewed in this paper.—Author’s Abstract

C57Bl/6 mice and mice deficient in the CD40 molecule were infected with three strains of Mycobacterium avium. Two of the M. avium strains proliferated more extensively in CD40-deficient (CD40−/−) mice than in control mice. The increased susceptibility to infection of CD40−/− mice was associated with the generation of poorer interleukin-12 (IL-12) p40 and interferon-gamma (IFN-gamma) responses as compared to the controls, suggesting a role for CD40 in the development of protective immunity. In contrast, direct triggering of CD40 on infected macrophages failed to induce any anti-mycobacterial activity in infected macrophages.—Authors’ Abstract


Thalidomide has been shown to be an effective treatment in various immunologic diseases such as Crohn’s disease and rheumatoid arthritis. Its major effect is thought to be mediated by the inhibition of TNF-alpha, but the exact mechanism of action is still uncertain. Recent observations could demonstrate that the induction of monocyte apoptosis is a common feature of a variety of anti-inflammatory agents. Therefore, we investigated the role of thalidomide on monocyte apoptosis. Treatment with thalidomide resulted in apoptosis of human peripheral blood monocytes in a time- and dose-dependent manner as demonstrated by annexin V staining. Monocyte apoptosis required the activation of caspases, as combined stimulation by thalidomide together with the broad caspase inhibitor benzylxoycarbonyl-Val-Ala-Asp-fluromethyl ketone markedly prevented monocyte cell death. Apoptosis was triggered by a CD95/CD95 ligand, TNF-RI, and TRAIL-R1 independent pathway with an inhibition of AKT-1 kinase and consecutive mitochondrial release of cytochrome c, followed by the proteolytic activation of initiator caspase-9 and effector caspase-3. Our data suggest that thalidomide-induced monocyte apoptosis is at least partially mediated by a mitochondrial signaling pathway and might contribute to the complex immunomodulatory properties of the drug.—Authors’ Abstract


The role of the Toll-like receptor (TLR)-2 in the generation of protective immunity to Mycobacterium avium was evaluated using gene-disrupted mice. TLR-2−/− mice were more susceptible than wild-type C57Bl/6 mice to M. avium strains that were able to proliferate in vivo before the development of protective immunity and mycobacteriostasis. In contrast, the elimination of non-virulent strains was not affected by the mutation. The generation of interferon-gamma (IFN-gamma)-producing T cells and the expression of the interleukin-12 p40 gene were reduced in TLR-2−/− deficient mice as compared to C57Bl/6 mice early during infection with M. avium strain 2447. The generation of protective CD4+ T cells was also compromised in the mutated mice as compared with the controls. Our data show that TLR-2 is required for optimal immunity against certain virulent M. avium strains.—Authors’ Abstract


Macrophage cell membranes were labeled with PKH26 and subsequently incubated with latex beads to generate phagosomes surrounded by a red-fluorescent membrane suitable for flow cytometry. Following cell disruption and partial purification of phagosomes, these vesicles were readily distinguished from both cell debris and free beads released from disrupted vacuoles. Flow cytometry analysis of phagosomes stained with specific mAbs and FITC-labeled secondary antibodies showed progressive acquisition of both Rab7 and LAMP-1 consistent with movement along the endocytic pathway. Alternatively, macrophages were preloaded with the lysosomal tracer FITC-dextran before membrane labeling with PKH and incubation with latex beads. Phagosome-lysosome fusion was then quantified on the basis of the colocalization of red and green signals. Using these flow cytometry-based systems, we showed that co-internalization of beads with lysates of Mycobacterium tuberculosis, but not lysates from the nonpathogenic organism Mycobacterium smegmatis, markedly decreased phagosome acquisition of Rab7 and LAMP-1 and vesicle fusion with FITC-dextran-loaded lysosomes. Inhibition of phagolysosome fusion could be attributed, at least in part, to the mycobacterial cell wall glycolipid lipoarabinomannan, and further analysis showed complete rescue of phagosome maturation when cells were pretreated with vitamin D3 before exposure to lipoarabinomannan. Moreover, the ability of vitamin D3 to reverse the phenotype of phagosomes in the presence of the glycolipid was completely abrogated by LY-294002, suggesting that vitamin D3 promotes phagolysosome fusion via a phosphoinositide 3-kinase signaling pathway. These findings establish a robust platform technology based on labeling of phagocyte cell membranes and flow cytometry capable of supporting broad-based screens to identify microbial and other bioactive compounds that influence phagosome biology.—Authors’ Abstract


Interspecies variations and mutations associated with rifampin resistance in rpoB of Mycobacterium allow for the simultaneous identification of rifampin-resistant Mycobacterium tuberculosis and nontuberculous mycobacteria by PCR-SSCP analysis and PCR-sequencing. One hundred and ten strains of rifampin-susceptible M. tuberculosis, 14 strains of rifampin-resistant M. tuberculosis, and four strains of the M. avium complex were easily identified by PCR-SSCP. Of another seven strains, which showed unique SSCP patterns, three were identified as rifampin-resistant M. tuberculosis and four as M. terrae complex by subsequent sequence analysis of their rpoB DNAs (306 bp). These results were concordant with those obtained by susceptibility testing, biochemical identification, and 16S rDNA sequencing.—Authors’ Abstract


A novel duplex PCR method that can amplify the 235- and 136-bp rpoB DNAs of Mycobacterium tuberculosis complex and nontuberculous mycobacteria (NTM), respectively, with two different sets of primers was used to differentially identify 44 reference strains and 379 clinical isolates of mycobacteria in a single-step assay. Showing 100% sensitivity and specificity, the duplex
PCR method could clearly differentiate *M. tuberculosis* complex and NTM strains. In addition, restriction fragment length polymorphism analysis and direct sequencing of the amplicon of NTM could be used to supplement species identification.—Authors’ Abstract


Pathogenesis by mycobacteria requires the exploitation of host-cell signalling pathways to enhance the intracellular survival and persistence of the pathogen. The disruption of these pathways by mycobacteria causes impaired maturation of phagosomes into phagolysosomes, modulates host-cell apoptotic pathways and suppresses the host immune response. This review highlights the strategies employed by mycobacteria to subvert host-cell signalling and identifies key molecules involved in these processes that might serve as potential targets for new antimycobacterial therapies.—Authors’ Abstract


A key issue for the study of tuberculosis is to understand why individuals infected with *Mycobacterium tuberculosis* (Mt) experience different clinical outcomes. To better understand the dynamics of Mt infection and immunity, we have previously developed a temporal mathematical model that qualitatively and quantitatively characterizes the cellular and cytokine control network during infection. In this work we extend that model to a two compartmental model to capture the important processes of cellular activation and priming that occur between the lung and the nearest draining lymph node. We are able to reproduce typical disease progression scenarios including primary infection, latency or clearance. Then we use the model to predict key processes determining these different disease trajectories (i.e., identify bifurcation parameters), suggesting directions for further basic science study and potential new treatment strategies.—Authors’ Abstract


The pathogenic mycobacteria are an insidious group of bacterial pathogens that cause the deaths of millions of people every year. One of the reasons these pathogens are so successful is that they are able to invade and replicate within host macrophages, one of the first lines of defence against intruding pathogens. In contrast, non-pathogenic mycobacteria, such as *Mycobacterium smegmatis* are killed rapidly by macrophages. In order to understand better the series of events that allow pathogenic mycobacteria to survive and replicate within macrophages, while the non-pathogenic mycobacteria are killed rapidly, we inoculated the human monocytic cell line U937 with pathogenic (*M. tuberculosis* and *M. avium*) and non-pathogenic (*M. smegmatis*) mycobacteria and monitored the expression of over 3500 genes at 4, 12 and 24 hr post-inoculation using a commercially available gene array system. We observed multiple differences in the gene expression patterns of monocytes infected with pathogenic and non-pathogenic mycobacteria including genes involved in cytokine, lymphokine and chemokine production, adhesion, apoptosis, signal transduction, transcription, protein cleavage, actin polymerization and growth. We also observed differences in gene expression profiles in monocytes infected with pathogenic and non-pathogenic mycobacteria including genes involved in cytokine, chemokine production, adhesion, apoptosis, signal transduction, transcription, protein cleavage, actin polymerization and growth. These results increase the understanding of the mechanisms used by pathogenic mycobacteria to cause disease, the host response to these organisms, and provide new insights for antimycobacterial intervention strategies.—Authors’ Abstract

Inducible nitric oxide synthase (iNOS) is a cytoplasmic protein responsible for the generation of nitric oxide (NO.) in macrophages. In this work, we hypothesized that the intracellular localization of iNOS is significant for effective delivery of NO. to phagosomes containing ingested microorganisms. Using immunofluorescence microscopy and Western blot analysis, iNOS was shown to localize in the vicinity of phagosomes containing latex beads in stimulated macrophages. iNOS also localized to phagosomes containing Escherichia coli. The colocalization of iNOS with ingested latex beads was an actin-dependent process, since treatment with the actin microfilament disrupter cytochalasin D prevented iNOS recruitment to latex bead phagosomes. In contrast to E. coli and inert particle phagosomes, mycobacterial phagosomes did not colocalize with iNOS. This study demonstrates that (i). iNOS can be recruited to phagosomes; (ii). this recruitment is dependent on a functional actin cytoskeleton; (iii). certain microorganisms have the ability to prevent or reduce colocalization with iNOS; and (iv). spatial exclusion of iNOS may play a role in Mycobacterium tuberculosis pathogenesis.—Authors’ Abstract


Advances are now being made in terms of understanding both the initiation of the adaptive or acquired response to tuberculosis infection and its interface with elements of the innate response, as well as much later events in terms of the chronic disease state where reactivation can potentially occur. Despite this, there are still several elements of the adaptive response that remain poorly understood.—Author’s Abstract


In the majority of individuals infected with Mycobacterium tuberculosis, the bacilli cause a long-term asymptomatic infection called latent tuberculosis, a state during which the bacilli reside within granulomas. Latently infected individuals have around 10% risk of progression to clinical disease at a later stage. Determining the state of the
mycobacteria and the host cells during this latent phase, i.e., within the granulomas, would greatly improve our understanding of the physiopathology of tuberculosis, and thus enable the development of new therapeutic means to treat the one-third of the world’s population who are latently infected. We have developed an in vitro model of human mycobacterial granulomas, enabling the cellular and molecular analysis of the very first steps in the host granulomatous response to either mycobacterial compounds or live mycobacterial species. In vitro mycobacterial granulomas mimic natural granulomas very well, with the progressive recruitment of macrophages around live bacilli or mycobacterial antigen-coated beads, their differentiation into multinucleated giant cells and epithelioid cells, and the final recruitment of a ring of activated lymphocytes. Besides morphological similarities, in vitro granulomas also functionally resemble natural ones, with the development of intense cellular co-operation and intracellular mycobactericidal activities.—Authors’ Abstract


In the rich, developed parts of the world there has been a steady and simultaneous increase in at least three groups of disease: (1) allergies, (2) inflammatory bowel diseases (IBD; e.g., Crohn’s disease and ulcerative colitis) and (3) autoimmunity (e.g., type 1 diabetes and multiple sclerosis). Because the medical world is so compartmentalised it was some time before the connection between these increases was noticed and understood. There is now evidence that the simultaneous increase in these diseases of immunodysregulation is at least partly attributable to malfunction of regulatory T cells (Treg). This paper provides an overview of relevant work in each of these fields of medicine (though with emphasis on the allergic disorders), and concludes that the increasing failure of Treg is a consequence of diminished exposure to certain micro-organisms that are “old friends,” because of their continuous presence throughout mammalian evolution. These organisms, which include saprophytic mycobacteria, helminths and lactobacilli, are recognised by the innate immune system as harmless, and as adjuvants for Treg induction. Polymorphisms of components of the innate immune system such as TLR2 and NOD2 appear to define subsets of the population that will develop immunoregulatory disorders when living in the modern environment. A further role of the “old friends” and of the Treg that they induce might be to maintain the levels of regulatory IL-10 secreting macrophages and antigen-presenting cells, which are depleted in asthma and Crohn’s disease. These concepts are leading to novel therapies based on harmless organisms or their components. Phase I/II clinical trials have yielded some statistically significant results, and phase II trials are in progress.—Authors’ Abstract


We found previously that immunosuppressive macrophages (Mphis) induced by Mycobacterium intracellulare infection (MI-Mphis) transmitted their suppressor signals to target T cells through cell contact with target T cells. In this study, we examined what kinds of Mphi surface molecules are required for such cell-to-cell interaction. First, it was found that a B7-1-like molecule (B7-1LM) recognizable with one of three test clones of anti-B7-1 monoclonal antibodies (mAbs) was required for expression of the Mphi suppressor activity. Neither anti-B7-2, anti-ICAM-1, nor anti-VCAM-1 mAb blocked the Mphi suppressor activity. Second, MI-Mphis increased the expression of B7-1LM in parallel with the acquisition of the suppressor activity. Neither anti-B7-2, anti-ICAM-1, nor anti-VCAM-1 mAb blocked the Mphi suppressor activity. Second, MI-Mphis increased the expression of B7-1LM in parallel with the acquisition of the suppressor activity. Moreover, MI-Mphis bound with target T cells in a B7-1LM-dependent fashion. Third, mAb blocking of CTLA-4 on target T cells did not reduce the suppressor activity of MI-Mphis,
suggesting the role of a putative molecule on target T cells other than CTLA-4 as the receptor for B7-1LM of MI-Mphis. Fourth, concanavalin A (Con A) stimulation of MI-Mphis was needed for effective cell contact with target T cells and subsequent expression of the suppressor activity of MI-Mphis. Fifth, the Con A-induced increase in the suppressor activity of MI-Mphis was inhibited by KN-62 but not by herbimycin A, H-7, nor H-88, indicating that Con A-induced up-regulation of MI-Mphi function is mediated by calmodulin-dependent protein kinase II or ATP/P2Z receptors, but independent of protein tyrosine kinase, protein kinase C, and protein kinase A. These findings indicate that a B7/CTLA-4-independent mechanism is needed for the transmission of the suppressor signals from MI-Mphis to target T cells.—Authors’ Abstract


The recent publication of the genome sequence of Mycobacterium bovis showed >99.95% identity to M. tuberculosis. No genes unique to M. bovis were found. Instead numerous single-nucleotide polymorphisms (SNPs) were identified. This has led to the hypothesis that differential gene expression due to SNPs might explain the differences between the human and bovine tubercle bacilli. One phenotypic distinction between M. tuberculosis and M. bovis is nitrate reduction, which not only is an essential diagnostic tool but also contributes to mycobacterial pathogenesis. We previously showed that narGHJI encodes a nitrate reductase in both M. tuberculosis and M. bovis and that NarGHJI-mediated nitrate reductase activity was substantially higher in the human tubercle bacillus. In the present study we used a genetic approach to demonstrate that an SNP within the promoter of the nitrate reductase gene cluster narGHJI is responsible for the different nitrate reductase activity of M. tuberculosis and M. bovis. This is the first example of an SNP that leads to differential gene expression between the human and bovine tubercle bacilli.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 420.


Chronic inflammation associated with cachexia, weight loss, fever and arthralgia is the hallmark of advanced mycobacterial diseases. These symptoms are attributed to the chronic stimulation of tumour necrosis factor (TNF)-alpha. Mycobacterial components directly stimulate adherent cells to secrete TNF-alpha. We have shown recently that IgG1 antimycobacterial antibodies play a role in augmenting TNF-alpha in purified protein derivative (PPD)-stimulated adherent cells from non-BCG-vaccinated donors. We now show that IgG1 antibodies can also augment TNF-alpha expression in stimulated adherent cells obtained from BCG-vaccinated donors and this augmentation is not linked to interleukin (IL)-10 secretion. In addition IgG1 antimycobacterial antibodies can reverse the effect of TNF-alpha blockers such as pentoxifylline and thalidomide. These studies therefore have clinical implications for anti-inflammatory drug treatments which are used increasingly to alleviate symptoms associated with chronic inflammation.—Authors’ Abstract

Torres, D., Barrier, M., Bihl, F., Quesniaux, V. J., Maillet, I., Akira, S., Ryffel, B., and Erard, F. Toll-like receptor 2 is required for optimal control of Listeria

The control of *Listeria monocytogenes* infection depends on the rapid activation of the innate immune system, likely through Toll-like receptors (TLR), since mice deficient for the common adapter protein of TLR signaling, myeloid differentiation factor 88 (MyD88), succumb to *Listeria* infection. In order to test whether TLR2 is involved in the control of infections, we compared the host response in TLR2-deficient mice with that in wild-type mice. Here we show that TLR2-deficient mice are more susceptible to systemic infection by *Listeria* than are wild-type mice, with a reduced survival rate, increased bacterial burden in the liver, and abundant and larger hepatic microabscesses containing increased numbers of neutrophils. The production of tumor necrosis factor, interleukin-12, and nitric oxide and the expression of the costimulatory molecules CD40 and CD86, which are necessary for the control of infection, were reduced in TLR2-deficient macrophages and dendritic cells stimulated by *Listeria* and were almost abolished in the absence of MyD88, coincident with the high susceptibility of MyD88-deficient mice to *in vivo* infection. Therefore, the present data demonstrate a role for TLR2 in the control of *Listeria* infection, but other MyD88-dependent signals may contribute to host resistance.—Authors’ Abstract


We examined the immunological abnormality in a patient with recurrent *Mycobacterium avium* infection. T cells from the patient showed decreased ability both to produce IFN-gamma and to proliferate in response to IL-12. Despite decreased expression of IL-12R beta1 and beta2 chains in the patient’s PHA-activated T cells, there was no difference in IL-12-induced tyrosine and serine phosphorylation of STAT4 in PHA-activated T cells between the patient and healthy subjects, suggesting that IL-12R signals are transmitted to STAT4 in the patient’s PHA-activated T cells. Using EMSA, confocal laser microscopy, and Western blotting, we demonstrated that the nuclear translocation of STAT4 in response to IL-12 is reduced in PHA-activated T cells from the patient when compared with those from healthy subjects. Leptomycin B was used to examine whether nuclear export of STAT4 is increased in the patient’s T cells. However, leptomycin B treatment did not reverse impaired IL-12-induced nuclear accumulation of STAT4. Although the exact mechanism responsible for the impaired STAT4 nuclear translocation in this patient remains unclear, the absence of mutation in the IL-12Rbeta1, IL-12Rbeta2, STAT4, and STAT4-binding sequence of the IFN-gamma gene and preservation of STAT4 tyrosine and serine phosphorylation suggest the existence of a defective STAT4 nuclear translocation. This defect is likely responsible for the impaired STAT4 nuclear translocation in IL-12-stimulated T cells, leading to impairment of both IFN-gamma production and cell proliferation. To the best of our knowledge, this is the first report of a patient with atypical mycobacterial infection associated with impairment of STAT4 nuclear translocation.—Authors’ Abstract


We expressed the CTL epitope of OVA (OVA(257–264)) in an acute (*Listeria* monocytogenes (LM)-OVA) and a chronic intracellular pathogen (*Mycobacterium bovis* (BCG)-OVA), to evaluate the kinetics of Ag presentation. LM-OVA proliferated rapidly *in vivo*, resulting in profound LM-OVA expansion within the first 24 hr of infection, culminating in the generation of a potent CD8+ T cell response, which peaked on day 7 but underwent a rapid attrition subsequently. In contrast, BCG-OVA exhibited reduced growth *in vivo*, resulting in a de-
layered CD8+ T cell response that increased progressively with time. Relative to LM-OVA, BCG-OVA induced persistently increased numbers of apoptotic (annexin V+) CD8+ T cells. Ag presentation in vivo was evaluated by transferring Thy1.2+ carboxyfluorescein-labeled OT1 transgenic CD8+ T cells into infected Thy1.1 + congenic recipient mice. LM-OVA induced rapid Ag presentation that was profound in magnitude, with most of the transferred cells getting activated within 4 days and resulting in a massive accumulation of activated donor CD8+ T cells. In contrast, Ag presentation induced by BCG-OVA was delayed, weaker in magnitude, which peaked around the second week of infection and declined to a low level subsequently. Increasing the dose of BCG-OVA while enhancing the magnitude of Ag presentation did not change the kinetics. Furthermore, a higher dose of BCG-OVA also accelerated the attrition of OVA(257–264)-specific CD8+ T cells. Relative to LM-OVA, the dendritic cells in BCG-OVA-infected mice were apoptotic for prolonged periods, suggesting that the rapid death of APCs may limit the magnitude of Ag presentation during chronic stages of mycobacterial infection.—Authors’ Abstract


OBJECTIVE: Tumor necrosis factor (TNF), an important inflammatory mediator in tuberculosis, has been implicated in causing accelerated HIV disease progression in HIV-associated tuberculosis. However, TNF blockade, particularly by monoclonal antibody, has been associated with the reactivation of latent Mycobacterium tuberculosis infection by the impairment of mycobacterial immunity. This phase 1 study examined the safety, microbiology, immunology, and virology of TNF blockade using etanercept (soluble TNF receptor, Enbrel) during the initial treatment of HIV-associated tuberculosis. DESIGN: A single-arm trial, with key endpoints compared with historical controls,
conducted in Mulago Hospital, Kampala, Uganda. SUBJECTS: Sixteen HIV-1-infected patients and 42 CD4-frequency-matched controls with sputum smear-positive tuberculosis and CD4 cell counts >200 cells/microl. INTERVENTION: Etanercept 25 mg, eight doses administered subcutaneously twice weekly beginning on day 4 of tuberculosis therapy. MAIN OUTCOME MEASURES: Serial examination, radiography, sputum culture, CD4 T-cell counts, plasma log10 HIV-RNA copy numbers. RESULTS: Trends towards superior responses to tuberculosis treatment were evident in etanercept-treated subjects in body mass, performance score, number of involved lung zones, cavitary closure, and time to sputum culture conversion. Etanercept treatment resulted in a 25% increase in CD4 cells by week 4 (p = 0.1 compared with controls). The change in CD4 cell count was inversely related to the change in serum neopterin, a marker of macrophage activation. There was no effect on plasma HIV RNA. CONCLUSION: Etanercept can be safely administered during the initial treatment of pulmonary tuberculosis. Further studies are warranted to examine the effects of etanercept on T-cell numbers, activation and apoptosis in AIDS and tuberculosis.—Authors’ Abstract


This study was undertaken to determine the value of incorporating fluorescence into cytopathological evaluation of lymph node fine-needle aspiration (FNA) specimens suspected of harboring mycobacterial species. The study population consisted of 1,044 HIV-positive and -negative patients referred for FNA to the cytopathology unit of a South African medical school located in a very high HIV prevalence region. Each aspirate was assessed on routine Papanicolaou-stained slides for morphologic characteristics of mycobacterial infection. The same glass slides were then viewed under fluorescent microscopy to determine the presence or absence of mycobacterial autofluorescence. Using multivariate analysis, results of both cytology and fluorescence were compared with mycobacterial culture as the final arbiter of the presence of organisms. In this large clinical study, compared with culture, cytomorphology showed sensitivity of 84.9%, but low specificity of only 50.9%. Fluorescence demonstrated lower sensitivity of 65.9%, but improved specificity of 73.0%. Taken together, positivity of both cytology and fluorescence improved specificity to 81.8%. Fluorescent microscopy is rapid, inexpensive, and cost-effective; neither radioactive materials nor further staining are required. It is felt that this methodology would be of diagnostic benefit if used on morphologically suspicious samples in areas with a high prevalence of HIV and mycobacterial infections. Appropriate therapy could be commenced within hours of FNA, with reduction in the current number of patients lost to follow-up while awaiting results of culture. The technique is readily extended to other FNA types such as deep organ aspirates. Autofluorescence of organisms specifically requires usage of Papanicolaou staining; the technique cannot be used in histopathologic specimens stained with hematoxylin-eosin.—Authors’ Abstract


Previous studies have shown the mitogen-activated protein kinases (MAPKs) to be activated in macrophages upon infection with Mycobacterium, and that expression of TNF-alpha and inducible NO synthase by infected macrophages was dependent on MAPK activation. Additional analysis demonstrated a diminished activation of p38 and extracellular signal-regulated kinase (ERK)1/2 in macrophages infected with pathogenic strains of Mycobacterium avium compared with infections with the fast-
growing, nonpathogenic *Mycobacterium smegmatis* and *Mycobacterium phlei*. However, the upstream signals required for MAPK activation and the mechanisms behind the differential activation of the MAPKs have not been defined. In this study, using bone marrow-derived macrophages from BALB/c mice, we determined that ERK1/2 activation was dependent on the calcium/calmodulin/calmodulin kinase II pathway in both *M. smegmatis* - and *M. avium*-infected macrophages. However, in macrophages infected with *M. smegmatis* but not *M. avium*, we observed a marked increase in cAMP production that remained elevated for 8 hr postinfection. This *M. smegmatis*-induced cAMP production was also dependent on the calmodulin/calmodulin kinase pathway. Furthermore, stimulation of the cAMP/protein kinase A pathway in *M. smegmatis*-infected cells was required for the prolonged ERK1/2 activation and the increased TNF-alpha production observed in these infected macrophages. Our studies are the first to demonstrate an important role for the calmodulin/calmodulin kinase and cAMP/protein kinase A pathways in macrophage signaling upon mycobacterial infection and to show how cAMP production can facilitate macrophage activation and subsequent cytokine production.—Authors’ Abstract

### Immunopathology, Leprosy


The prevalence of various autoantibodies was studied in 75 leprosy patients comprising eight patients with lepromatous leprosy (LL), 36 patients with borderline lepromatous leprosy (BL) and 31 patients with borderline tuberculoid leprosy (BT), along with 100 normal controls. Certain autoantibodies such as anti-nuclear antibodies (ANA), antisecond stranded DNA (anti-ssDNA) and anti-neutrophil cytoplasmic antibodies (ANCA) were raised among leprosy patients. When ANCA specificities to anti-myeloperoxidase (anti-MPO), anti-proteinase3 (anti-PR3) and anti-lactoferrin (anti-LF) were studied, it was found that the patterns of immunofluorescence such as perinuclear (p-ANCA), cytoplasmic (c-ANCA) and atypical (X-ANCA) and specificity by ELISA to anti-MPO, anti-PR3 and anti-LF varied in the LL, BL and BT groups. However, a higher amount of c-ANCA was observed in 62.5% of leprosy cases, while the incidences of p-ANCA and X-ANCA were lower. The LL group showed a higher incidence of autoantibodies as compared with the BL and BT groups, along with a male preponder-


In this study we looked for the presence of antibodies to cardiolipin, cerebrosides, and whole lipids extracted from *M. leprae*, *M. tuberculosis* and *M. habana*, in the serum of patients with clinically cured lepromatous leprosy (sixteen) or tuberculosis (sixteen), 8 to 12 months after arresting the corresponding multi-drug therapy (MDT). Compared to healthy controls (sixteen), both leprosy and tuberculosis ex-patients had still significant levels of antibodies to the three mycobacterial lipids but no detectable levels of antibodies to cardiolipin or cerebroside...
lipids. Although leprosy and tuberculosis sera recognized the homologous mycobacterial lipids in a preferential fashion, all of them, on the average, reacted more strongly with the lipids of \textit{M. habana}. This observation backs up, in a certain way, the proposition of using \textit{M. habana} as a prospective vaccine for leprosy and tuberculosis.—Authors’ Abstract


BACKGROUND: Nerve damage is a common and disabling feature of leprosy, with unclear aetiology. It has been reported that the peroxidizing agents of myelin lipids-nitric oxide (NO) and peroxynitrite-are produced in leprosy skin lesions. OBJECTIVES: To investigate the localization of nitrotyrosine (NT)—a local end-product of peroxynitrite—in leprosy lesions where dermal nerves are affected by a granulomatous reaction. METHODS: We investigated by immunohistochemistry and immunoelectron microscopy the localization of the inducible NO synthase (iNOS) and NT in biopsies exhibiting dermal nerves from patients with untreated leprosy. RESULTS: There were abundant NT-positive and iNOS-positive macrophages in the borderline leprosy granulomas infiltrating peripheral nerves identified by light microscopy, S-100 and neurofilament immunostaining. Immunoelectron microscopy showed NT reactivity in neurofilament aggregates and in the cell wall of \textit{Mycobacterium leprae}. CONCLUSIONS: Our results suggest that NO and peroxynitrite could be involved in the nerve damage following borderline leprosy.—Authors’ Abstract


This is a blinded, retrospective, correlative study of classification of leprosy by cytology, clinical examination, and bacterial density. One hundred consecutive adequate aspirates from skin lesions of leprosy were studied. The Ridley-Jopling (R-J) five-group classification system was used. May-Gruenwald-Giemsa (MGG) and Ziehl-Neelsen (Z-N) stains were employed. Complete clinical, cytological, and bacteriological concordance was found in 88 patients. One-step mismatch in classification was seen in 12 patients with cytomorphological features of borderline-borderline (BB/mid-borderline) leprosy. Cytomorphological features of BB leprosy in aspirates from skin lesions should alert the cytopathologist to the possibility that the bacteriological index (BI) may vary widely. Appropriate steps must be taken to ensure accurate reporting of BI.—Authors’ Abstract


Leprosy is a chronic infectious disease caused by \textit{Mycobacterium Leprae} and is characterized by well-recognized pathological changes. But there are various disagreement in clinical type and histological finding of leprosy. We observed highest parity in LL and TT group followed by histoid, BT, BL, BB, & indeterminate respectively. There was 10% minor disagreement (difference of one group) and 5% major disagreement (difference of two or more group). Non-specific histological finding was present in 20% cases.—Authors’ Abstract


OBJECTIVE: To determine whether addition of low dose prednisolone to multidrug treatment can prevent reaction and nerve
function impairment in leprosy. DESIGN: Multicenter, double blind, randomized, placebo controlled, parallel group trial. SETTING: Six centers in Bangladesh and Nepal. PARTICIPANTS: 636 people with newly diagnosed multibacillary leprosy. INTERVENTION: Prednisolone 20 mg/day for three months, with tapering dose in month 4, plus multidrug treatment, compared with multidrug treatment alone. MAIN OUTCOME MEASURES: Signs of reaction, impairment of sensory and motor nerve function, and nerve tenderness needing full dose prednisolone at four months and one year. RESULTS: Prednisolone had a significant effect in the prevention of reaction and nerve function impairment at four months (relative risk 3.9, 95% confidence interval 2.1 to 7.3), but this was not maintained at one year (relative risk 1.3, 0.9 to 1.8). Fewer events occurred in the prednisolone group at all time points up to 12 months, but the difference at 12 months was small. Subgroup analysis showed a difference in response between people with and without impairment of nerve function at diagnosis. CONCLUSIONS: The use of low dose prophylactic prednisolone during the first four months of multidrug treatment for leprosy reduces the incidence of new reactions and nerve function impairment in the short term, but the effect is not sustained at one year. The presence of nerve function impairment at diagnosis may influence the response to low dose prednisolone.—Authors’ Abstract


Culture filtrate protein 10 (CFP-10) from Mycobacterium tuberculosis is a well-characterized immunodominant 10-kDa protein antigen known to elicit a very potent early gamma interferon response in T cells from M. tuberculosis-infected mice and humans. The sequence of the Mycobacterium leprae homologue of CFP-10 shows only 40% identity (60% homology) at the protein level with M. tuberculosis CFP-10 and thus has the potential for development as a T- or B-cell reactive antigen for specific diagnosis of leprosy. Antisera raised in mice or rabbits against recombinant M. leprae and M. tuberculosis CFP-10 proteins reacted only with homologous peptides from arrays of overlapping synthetic peptides, indicating that there was no detectable cross-reactivity at the antibody level. Sera from leprosy and tuberculosis patients were also specific for the homologous protein or peptides and showed distinct patterns of recognition for either M. leprae or M. tuberculosis CFP-10 peptides. At the cellular level, only 2 of 45 mouse T-cell hybridomas raised against either M. leprae or M. tuberculosis CFP-10 displayed a cross-reactive response against the N-terminal heterologous CFP-10 peptide, the region that exhibits the highest level of identity in the two proteins; however, the majority of peptide epitopes recognized by mouse T-cell hybridomas specific for each protein did not cross-react with heterologous peptides. Coupled with the human serology data, these results raise the possibility that peptides that could be used to differentiate infections caused by these two related microorganisms could be developed. Immunohistochemical staining of sections of M. leprae-infected nude mouse footpads resulted in strongly positive staining in macrophages and dendritic cells, as well as weaker staining in extracellular areas, suggesting that M. leprae CFP-10, like its homologue in M. tuberculosis, is a secreted protein.—Authors’ Abstract


Macrophages are decisive cells for the course of leprosy as they phagocyte Mycobacterium leprae and have the potential to influence the specific immune response. Expression and release of the myeloid-related
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protein (MRP) 8 and MRP14 (S100A8 and S100A9) characterize a proinflammatory subtype of macrophage that is prominent in, for example, murine infection with lack of a T helper 1 cell response and in certain highly active chronic inflammations of mice and humans. We investigated cutaneous biopsies of the different forms of leprosy (41 untreated patients) including leprosy reaction type 1 (reversal reaction) and type 2 (erythema nodosum leprosum) (N = 18) for expression of MRP8 and MRP14 by subtypes of macrophages. Concomitantly we determined serum levels of MRP8 and MRP14 by sandwich enzyme-linked immunosorbent assay. Expression of MRP8 and MRP14 by CD68-positive macrophages was low in tuberculoid leprosy and rose significantly in borderline tuberculoid leprosy and especially in multibacillary forms, there being expressed by mycobacteria-loaded foam cells. A significant rise of MRP8 and MRP14 expression also occurred in lepra reactions compared to the corresponding non-reactional forms. In type 2 reactions this additional increase was associated with a significant elevation of serum levels. In type 1 it was associated with expression of MRP8 and MRP14 by epitheloid and giant cells, which so far were considered not to express both proteins. In conclusion, we present evidence that the two prominent proteins MRP8 and MRP14 can be re-expressed in vivo by tissue macrophages in chronic infection, that their increased expression is characteristic for a macrophage subtype associated with high inflammatory but low antimycobacterial activity in the absence of a T helper 1 response, and that their significant rise in serum during erythema nodosum leprosum bears diagnostic and pathophysiological relevance.—Authors’ Abstract


Mycobacterium leprae, the causative agent of leprosy invades Schwann cells of the peripheral nerves leading to nerve damage and disfigurement, which is the hallmark of the disease. Wet experiments have shown that M. leprae binds to a major peripheral nerve protein, the myelin P zero (P0). This protein is specific to peripheral nerve and may be important in the initial step of M. leprae binding and invasion of Schwann cells which is the feature of leprosy. Though the receptors on Schwann cells, cytokines, chemokines and antibodies to M. leprae have been identified the molecular mechanism of nerve damage and neurodegeneration is not clearly defined. Recently pathogen and host protein/nucleotide sequence similarities (molecular mimicry) have been implicated in neurodegenerative diseases. The approach of the present study is to utilise bioinformatic tools to understand leprosy nerve damage by carrying out sequence and structural similarity searches of myelin P0 with leproma and other genomic database. Since myelin P0 is unique to peripheral nerve, its sequence and structural similarities in other neuropathogens have also been noted. Comparison of myelin P0 with the M. leprae proteins revealed two characterised proteins, Ferrodoxin NADP reductase and a conserved membrane protein, which showed similarity to the query sequence. Comparison with the entire genomic database (www.ncbi.nlm.nih.gov) by basic local alignment search tool for proteins (BLASTP) and fold classification of structure-structure alignment of proteins (FSSP) searches revealed that myelin P0 had sequence/structural similarities to the poliovirus receptor, coxsackie-adenovirus receptor, anthrax protective antigen, diphtheria toxin, herpes simplex virus, HIV gag-1 peptide, and gp120 among others. These proteins are known to be associated directly or indirectly with neurodegeneration. Sequence and structural similarities to the immunoglobin regions of myelin P0 could have implications in host-pathogen interactions, as it has homophilic adhesive properties. Although these observed similarities are not highly significant in their percentage identity, they could be functionally important in molecular mimicry, receptor binding and cell signaling events involved in neurodegeneration.—Authors’ Abstract

Granulomas, focal accumulations of immune cells, form in the lung during Mycobacterium tuberculosis infection. Chemokines, chemotactic cytokines, are logical candidates for inducing migration of T lymphocytes and monocytes to and within the lung. TNF influences chemokine expression in some models. TNF-deficient mice infected with M. tuberculosis are highly susceptible to disease, and granuloma formation is inhibited. Through in vitro assays, we demonstrate that neutralization of TNF in M. tuberculosis-infected macrophages led to a reduction in many inflammatory chemokines, such as C-C chemokine ligand 5, CXC ligand 9 (CXCL9), and CXCL10. In TNF-deficient mice, immune cells migrated to the lungs early after infection, but did not organize to form granulomas within the lung. Although chemokine expression, as measured in whole lung tissue, was not different, the expression of chemokines in the CD11b(+) subset of cells isolated ex vivo from the lungs of TNF-deficient mice had reduced expression of C-C chemokine ligand 5, CXCL9, and CXCL10 at early time points after TNF neutralization. Local expression of CXCR3-binding chemokines within the lungs, as determined by in situ hybridization, was also affected by TNF. Therefore, TNF affects the expression of chemokines by macrophages in vitro and CD11b(+) cells in vivo, which probably influences the local chemokine gradients and granuloma formation.—Authors’ Abstract


We report that stimulation of Mycobacterium tuberculosis (M. tuberculosis) secretory antigen (MTSA)-differentiated dendritic cells (DCs) and MTSA-matured DCs with M. tuberculosis cell extract (CE) down-regulated proinflammatory responses to CE-primed T (CE-T) cells by increasing surface expression of CD86 after CE stimulation. CE stimulation also decreased interleukin (IL)-12p40 and interferon (IFN)-gamma levels and increased IL-10 and transforming growth factor-beta 1 (TGF-beta 1) levels from these DCs. Blocking either CD86, IL-10, or TGF-beta with monoclonal antibodies before CE stimulation restored the attenuated T helper 1 (Th1) responses of CE-T cells. Conversely, treatment of these DCs with IL-12p70 and/or IFN-gamma completely restored Th1 responses from CE-T cells. These results indicate that M. tuberculosis secretory antigens down-regulate proinflammatory Th1 responses to mycobacteria by differentially modulating the cytokine profiles and surface densities of costimulatory molecules on DCs. Of importance, this down-regulation is independent of the maturation status of MTSA-activated DCs and can be rescued after treatment of DCs with IFN-gamma or IL-12.—Authors’ Abstract


The currently used method for immunological detection of tuberculosis infection, the tuberculin skin test, has low specificity. Antigens specific for Mycobacterium tuberculosis to replace purified protein derivative are therefore urgently needed. We have performed a rigorous assessment of the diagnostic potential of four recently identified antigens (Rv2653, Rv2654, Rv3873, and
Rv3878) from genomic regions that are lacking from the Mycobacterium bovis bacillus Calmette-Guerin (BCG) vaccine strains as well as from the most common nontuberculous mycobacteria. The fine specificity of potential epitopes in these molecules was evaluated by sensitive testing of the T-cell responses of peripheral blood mononuclear cells derived from M. bovis BCG-vaccinated healthy individuals to synthesized overlapping peptides. Three of the four molecules contained regions with significant specificity problems (Rv2653, Rv3873, and Rv3878). We selected and combined the specific peptide stretches from the four proteins not recognized by M. bovis BCG-vaccinated individuals. These peptide stretches were tested with peripheral blood mononuclear cells obtained from patients with microscopy- or culture-confirmed tuberculosis and from healthy M. bovis BCG-vaccinated controls. The combination of the most promising stretches from this analysis showed a sensitivity level (57%) comparable to the level found with the two well-known M. tuberculosis-specific proteins ESAT-6 and CFP-10 (75 and 66%, respectively). The combination of ESAT-6, CFP-10, and the novel specific peptide stretches gave an overall sensitivity of 84% at a specificity of 97%. In a validation experiment with new experimental groups, the sensitivities obtained were 57% for the combination of peptides and 90% for the combination of the peptides, ESAT-6, and CFP-10. This combination gave a specificity of 95%.

Authors’ Abstract

Chandra, G., Selvaraj, P., Jawahar, M. S., Banurekha, V. V., and Narayanan, P. R.

Immune responses are elicited through antigen presentation and recognition by macrophages and T-lymphocytes, respectively. The immunomodulatory effect of vitamin D(3) on macrophage phagocytic potential with live Mycobacterium tuberculosis, spontaneous and M. tuberculosis culture filtrate antigen induced lymphocyte responses were studied in pulmonary tuberculosis patients (PTBPs) (N = 31) and normal healthy subjects (NHSs) (N = 43). Vitamin D(3) at a concentration of 10(–7) M significantly enhanced the macrophage phagocytosis of live M. tuberculosis in normal subjects with low phagocytic potential (less than 10%) (p = 0.015). No such increase was observed in PTBPs. Vitamin D(3) significantly decreased the spontaneous lymphoproliferative response (p = 0.022) and increased the apoptosis of peripheral blood mononuclear cells in PTBPs (p = 0.024). In normals, vitamin D(3) increased the spontaneous lymphoproliferative response. An inverse correlation between macrophage phagocytosis and spontaneous response was observed in NHSs, whereas a direct correlation was seen between vitamin D(3)-treated cells in normal subjects under in vitro condition. Vitamin D(3) decreased the M. tuberculosis culture filtrate antigen induced lymphocyte response significantly in normal subjects (p = 0.0003), while it had no influence on the lymphocyte response in PTBPs. The present study suggests that exposure to vitamin D(3) increases the phagocytic potential and spontaneous lymphoproliferative response but brings down the antigen-induced response in normals. In tuberculosis, addition of vitamin D(3) has no significant effect on antigen-induced lymphoproliferative response. This may be due to the unresponsive nature of the cells to the action of vitamin D(3) by virtue of the disease, which renders them inactive.—Authors’ Abstract


AIM: To analyse biological properties of Mycobacterium tuberculosis polypeptide antigen(Mtb-Ag) that could stimulate the proliferation of human gammadeltaT cells. METHODS: Mtb secretory proteins and Mtb-Ag treated via dialysis or with pronase were used to stimulate human PBMCs. After being cultured for 10 days, the phenotype of responsive cells was analyzed by
flow cytometry. **RESULTS:** The proliferation stimulating activity to human gammadeltaT cells of dialysed Mtb-Ag did not decrease remarkably, whereas that of the pronase-treated Mtb-Ag reduced significantly. Moreover, the proliferative stimulating activity to gammadeltaT cells of Mtb-Ag was notably higher than Mtb secretory protein. **CONCLUSION:** The component in Mtb-Ag that can stimulate the proliferation of human gammadeltaT cells is a non-secretory and protease-sensitive polypeptides with M(r) over 10,000.—Authors’ Abstract


The mycobacterial cell wall component lipoarabinomannan (LAM) has been described as a virulence factor of *Mycobacterium tuberculosis*, and modification of the terminal arabinan residues of this compound with mannose caps (producing mannosyl-capped LAM [ManLAM]) in *M. tuberculosis* or with phosphoinositol caps (producing phosphoinositol-capped LAM [PILAM]) in *Mycobacterium smegmatis* has been implicated in various functions associated with these lipoglycans. A structure-function analysis was performed by using LAMs and their biosynthetic precursor lipomannans (LMs) isolated from different mycobacterial species on the basis of their capacity to induce the production of interleukin-12 (IL-12) and/or apoptosis of macrophage cell lines. Independent of the mycobacterial species, ManLAMs did not induce IL-12 gene expression or apoptosis of macrophages, whereas PILAMs induced IL-12 secretion and apoptosis. Interestingly, uncapped LAM purified from *Mycobacterium chelonae* did not induce IL-12 secretion or apoptosis. Furthermore, LMs, independent of their mycobacterial origins, were potent inducers of IL-12 and apoptosis. The precursor of LM, phosphatidyl-myo-inositol dimannoside, had no activity, suggesting that the mannan core of LM was required for the activity of LM. The specific interaction of LM with Toll-like receptor 2 (TLR-2) but not with TLR-4 suggested that these responses were mediated via the TLR-2 signaling pathway. Our experiments revealed an important immunostimulatory activity of the biosynthetic LAM precursor LM. The ratio of LAM to LM in the cell wall of mycobacteria may be an important determinant of virulence, and enzymes that modify LM could provide targets for development of antituberculosis drugs and for derivation of attenuated strains of *M. tuberculosis.*—Authors’ Abstract


Antigens and mitogens have the innate ability to trigger cell proliferation and apoptosis thus exhibiting a dual-signal phenomenon. This dual-signal hypothesis was tested with mycobacterial antigens (PPD and heat killed *Mycobacterium tuberculosis*—MTB) in tuberculous pleuritis patients where the immune response is protective and compartmentalized. We compared and correlated the cell-cycle analysis and antigen-induced apoptosis in normal and patients’ peripheral blood mononuclear cells (PBMCs) and patients’ pleural fluid mononuclear cells (PFMCs). In cell-cycle analysis, PFMCs showed good mitotic response with PPD and MTB antigens where 10% and 7% of resting cells entered the S and G2/M phases of cell cycle, respectively. This antigen-induced proliferation of PFMCs correlated well with the lymphocyte transformation test (LTT) results. On the other hand, PFMCs also showed 21% of spontaneous apoptosis, which further increased to 43%, by induction with known apoptotic agent like Dexamethasone (DEX) and the mycobacterial antigens PPD and MTB. Further we demonstrated by anti-CD3 induction experiments that prior activation of cells is prerequisite for them to undergo apoptosis. Our results showed that PPD and MTB antigens induced both cell proliferation and apoptosis in PFMCs, which were pre-sensitized to mycobacterial antigens *in vivo.*
Thus the dual-signal phenomenon was operative against these antigens in tuberculous pleuritis. We also demonstrated that the activated cells are more predisposed to apoptosis.—Authors’ Abstract


The majority of healthy individuals exposed to Mycobacterium tuberculosis will not develop disease and identifying what constitutes ‘protective immunity’ is one of the holy grails of M. tuberculosis immunology. It is known that IFN-gamma is essential for protection, but it is also apparent that IFN-gamma levels alone do not explain the immunity/susceptibility dichotomy. The controversy regarding correlates of immunity persists because identifying infected but healthy individuals (those who are immune) has been problematic. We have therefore used recognition of the M. tuberculosis virulence factor early secretory antigenic target 6 to identify healthy, but infected individuals from tuberculosis (TB)-endemic and nonendemic regions (Ethiopia and Denmark) and have compared signals for cytokines expressed directly *ex vivo* with the pattern found in TB patients. We find that TB patients are characterized by decreased levels of Th1 cytokines and increased levels of IL-10 compared with the healthy infected and noninfected community controls. Interestingly, the healthy infected subjects exhibited a selective increase of message for the IL-4 antagonist, IL-4delta2, compared with both TB patients or noninfected individuals. These data suggest that long-term control of M. tuberculosis infection is associated not just with elevated Th1 responses but also with inhibition of the Th2 response.—Authors’ Abstract


Mycobacterium tuberculosis overcomes macrophage bactericidal activities and persists intracellularly. One mechanism by which M. tuberculosis avoids macrophage killing might be through inhibition of IFN-gamma-mediated signaling. In this study we provide evidence that at least two distinct components of M. tuberculosis, the 19-kDa lipoprotein and cell wall peptidoglycan (contained in the mycolylarabinogalactan peptidoglycan (mAGP) complex), inhibit macrophage responses to IFN-gamma at a transcriptional level. Moreover, these components engage distinct proximal signaling pathways to inhibit responses to IFN-gamma: the 19-kDa lipoprotein inhibits IFN-gamma signaling in a Toll-like receptor (TLR)2-dependent and myeloid differentiation factor 88-dependent fashion whereas mAGP inhibits independently of TLR2, TLR4, and myeloid differentiation factor 88. In addition to inhibiting the induction of specific IFN-gamma responsive genes, the 19-kDa lipoprotein and mAGP inhibit the ability of IFN-gamma to activate murine macrophages to kill virulent M. tuberculosis without inhibiting production of NO. These results imply that inhibition of macrophage responses to IFN-gamma may contribute to the inability of an apparently effective immune response to eradicate M. tuberculosis.—Authors’ Abstract


Alveolar macrophages constitute a primary defense against Mycobacterium tuberculosis, but they are unable to control M. tuberculosis without acquired T-cell immu-
This study determined the antigen-presenting cell function of murine alveolar macrophages and the ability of the model mycobacterium, *Mycobacterium bovis* BCG, to modulate it. The majority (80 to 85%) of alveolar macrophages expressed both CD80 (B7.1) and CD11c, and 20 to 30% coexpressed major histocompatibility complex II (MHC-II). Gamma interferon (IFN-gamma) enhanced MHC-II but not B7.1 expression. Naive or IFN-gamma-treated alveolar macrophages did not express CD86 (B7.2), CD11b, Mac-3, CD40, or F4/80. *M. bovis* BCG and the 19-kDa mycobacterial lipoprotein inhibited IFN-gamma-regulated MHC-II expression on alveolar macrophages, and inhibition was dependent on Toll-like receptor 2. The inhibition of MHC-II expression by the 19-kDa lipoprotein was associated with decreased presentation of soluble antigen to T cells. Thus, susceptibility to tuberculosis may result from the ability of mycobacteria to interfere with MHC-II expression and antigen presentation by alveolar macrophages.—Authors’ Abstract


Opportunistic infections such as pulmonary tuberculosis (TB) increase local HIV-1 replication and mutation. As AIDS progresses, alteration of the HIV-1 gp120 V3 sequence is associated with a shift in viral coreceptor use from CCR5 (CD195) to CXCR4 (CD184). To better understand the effect of HIV/TB coinfection, we screened transcripts from bronchoalveolar lavage cells with high density cDNA arrays and found that CXCR4 mRNA is increased in patients with TB. Surprisingly, CXCR4 was predominately expressed on alveolar macrophages (AM). *Mycobacterium tuberculosis* infection of macrophages in vitro increased CXCR4 surface expression, whereas amelioration of disease reduced CXCR4 expression in vivo. Bronchoalveolar lavage fluid from TB patients had elevated levels of CCL4 (macrophage inflammatory protein-1beta), CCL5 (RANTES), and CX3CL1 (fractalkine), but not CXCL12 (stromal-derived factor-1alpha). We found that *M. tuberculosis* infection of macrophages in vitro increased viral entry and RT of CXCR4, using HIV-1, but not of CCR5, using HIV-1. Lastly, HIV-1 derived from the lung contains CD14, suggesting that they were produced in AM. Our results demonstrate that TB produces a permissive environment for replication of CXCR4-using virus by increasing CXCR4 expression in AM and for suppression of CCR5-using HIV-1 by increasing CC chemokine expression. These changes explain in part why TB accelerates the course of AIDS. CXCR4 inhibitors are a rational therapeutic approach in HIV/TB coinfection.—Authors’ Abstract


To clarify what kinds of proteinases are secreted into the foci of allergic-inflammation involving delayed-type hypersensitivity reaction, we examined the characteristic releases of various proteinases into the foci of *Mycobacterium tuberculosis* (*M. tuber*.)-induced delayed-type allergic-inflammation in mice. The significant activities of cathepsin B and prolylendopeptidase were observed in the washing-fluids of subcutaneous inflammatory foci of *M. tuber*.-induced delayed-type allergic-inflammation in mice. The SDS-resistant complex of cathepsin B and a protein substrate with apparent molecular mass of 74 kDa was observed by Western blot analysis. On the other hand, no significant accumulations of other proteinases, such as matrix metalloproteinases, cathepsin D, and serine proteinases, were determined. CA-074, a specific inhibitor of cathepsin B, suppressed both swelling and cathepsin B activity in the footpad having *M. tuber*.-
induced delayed-type allergic-inflammation in vivo. These results suggest that cathepsin B may play an important role in the formation of M. tuber.-induced delayed-type allergic-inflammation.—Authors’ Abstract


The effect of purified mouse serum amyloid P-component (SAP) treatment of mouse alveolar macrophages (AMs) on their uptake of Mycobacterium tuberculosis Erdman was investigated, in vitro. SAP (0.5–50.0 micro g/ml), in a concentration-dependent manner, inhibited the M. tuberculosis uptake by the AMs; maximum inhibition (33.43%) occurred at 10.0 micro g/ml. The inhibition of uptake could be observed as early as 30 min after the incubation of AMs with 10.0 micro g/ml SAP; however, an incubation of 60 min induced maximum inhibition beyond which the response became static. The SAP-mediated decreased uptake of M. tuberculosis also resulted in their reduced intramacrophage growth as determined by colony-forming unit counts. SAP inhibited the uptake of mycobacteria in the presence of Ca(2+), and at pH = 5.6, the inhibition was abrogated. Deglycosylation of purified SAP with N-glycanase, and not with O-glycanase, blocked the SAP-mediated inhibition of the uptake. Heat-inactivated (80 degrees C; 1 hr; pH 7.0) SAP did not inhibit the uptake of M. tuberculosis by AMs. These data, apparently for the first time, indicate that purified mouse SAP, in a divalent cation- and N-linked oligosaccharide glycosylation-dependent manner, inhibited the in vitro uptake of M. tuberculosis Erdman by mouse AMs, which was also associated with their reduced intracellular growth.—Authors’ Abstract


Interferon (IFN)-gamma plays an essential role in host defense against infection with Mycobacterium tuberculosis, and its synthesis is critically regulated by interleukin (IL)-12, IL-18 and the recently identified IL-23. The present study was designed to determine the roles of these cytokines in IFN-gamma-mediated host defenses against M. tuberculosis. For this purpose, we compared host protective responses in IL-12p40 and IL-18 double-knockout (DKO) mice (which lacked both IL-12/IL-18 and also IL-23) and IFN-gamma gene-disrupted (GKO) mice. DKO mice were more resistant to the infection than GKO mice, as indicated by their extended survival and reduced live colony numbers in spleen, liver and lung. IFN-gamma was detected by ELISA in liver and lung homogenates, but not in spleen and serum, and in all organs by RT-PCR in DKO mice at comparable or reduced levels to those in wild-type mice. IFN-gamma production was reduced by depletion of CD4+ T cells, but not of natural killer (NK), NKT, gammadeltaT and dendritic cells. Neutralization of IFN-gamma or TNF-alpha by specific monoclonal antibodies (mAbs) significantly shortened the survival time of the infected DKO mice. Furthermore, anti-TNF-alpha mAb partially attenuated IFN-gamma synthesis in the liver of these mice. Finally, the expression level of inducible nitric oxide synthase (iNOS) mRNA in the spleen, liver and lung was considerable in DKO mice but only marginal or undetected in GKO mice. Our results indicate the presence of IL-12-, IL-18- and IL-23-independent host protective responses against mycobacterial infection mediated by IFN-gamma, which was secreted from helper T cells.—Authors’ Abstract

The tuberculin skin test for diagnosing *Mycobacterium tuberculosis* infection suffers from antigenic cross-reactivity of purified protein derivative with BCG, resulting in poor specificity in BCG-vaccinated populations. Comparative genomics has identified several genetic regions in *M. tuberculosis* and *M. bovis* that are deleted in *M. bovis* BCG. Proteins encoded in these regions will form the basis of new specific T-cell-based blood tests that do not cross-react with BCG, but only two, early secretory antigen target 6 and culture filtrate protein 10, have been studied in detail in humans. We investigated four novel gene products, encoded by RD2 (Rv1989c) and RD1 (Rv3873, Rv3878, and Rv3879c), that are absent from most or all of the vaccine strains of BCG, respectively. Sixty-seven overlapping peptides were tested in *ex vivo* gamma interferon enzyme-linked immunospot assays in 49 patients with culture-confirmed tuberculosis and 38 healthy BCG-vaccinated donors. Forty-five percent (95% confidence interval [CI], 31 to 57%) and 53% (95% CI, 39 to 67%) of the tuberculosis patients responded to Rv3879c and Rv3873, respectively, identifying these proteins as major *M. tuberculosis* T-cell antigens in humans, while 35 and 25% of the patients responded to Rv3878 and Rv1989c, respectively. Of the 38 BCG-vaccinated donors, 1 (2.6%) responded to peptides from Rv3878 and Rv3879c, 3 (7.9%) responded to Rv3873, and none responded to Rv1989c. Exclusion of cross-reactive peptides encoded in conserved motifs of Rv3873, a PPE family member, increased its specificity to 97.4%. The high specificity of Rv3879c peptides and nonconserved Rv3873 sequences, together with their moderate sensitivity in tuberculosis patients, identifies these peptides as candidates for inclusion in new T-cell-based tests for *M. tuberculosis* infection.—Authors’ Abstract

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Genetic variation influences immune responses and may contribute to differential development of tuberculosis (TB), particularly in immunosuppressed individuals. To examine the risk of *Mycobacterium tuberculosis* infection progressing to disease in the context of *M. tuberculosis*/human immunodeficiency virus (HIV) type 1 coinfection, HIV-1 RNA load and human leukocyte antigen (HLA) genotypes were determined among subjects from Harare, Zimbabwe, an area where both TB and HIV-1 are endemic. Patients with TB were compared with control subjects, stratified by HIV-1 infection status and progression of TB disease. Alleles of class I HLA-A and -C were associated with risk of developing active TB, depending on HIV-1 status. Among HIV-positive subjects, HIV-1 load was independently associated with increased risk of developing pulmonary TB. HLA DRB1 homozygosity among HIV-positive subjects was associated with reduced risk of developing pulmonary TB but increased risk of rapid progression to pleural effusion TB. These observations suggest that HLA plays a role in risk of developing symptomatic TB at various stages of disease and that these effects are modified by HIV-1 coinfection.—Authors’ Abstract

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Alveolar macrophages (AM) are the first professional phagocytes encountered by aerosols containing infections in the lungs, and their phagocytic capacity may be affected by these infections or environmental particles. The aim of this study was to evaluate the innate endocytic and phagocytic properties of human AM obtained from patients with pulmonary tuberculosis and to characterize the vacuoles in which *Mycobacterium tuberculosis* bacilli reside *in vivo*. AM were obtained by bronchoalveolar lavage from patients with suspected tuberculosis and from asymptomatic volunteers (controls). Clinical case definitions were based on mycobacterial culture
of respiratory specimens and HIV serology. To assess phagocytosis, endocytosis, and acidification of the endosomal system, AM were cultured with IgG-coated polystyrene beads, dextran, and a pH-sensitive reporter (3-(2,4-dinitroanilino)-3-amino-N-methylidipropylamine) and were evaluated by light and immunoelectron microscopy. Cells from 89 patients and 10 controls were studied. We found no significant difference between the two groups in the ability of AM either to ingest beads and dextran or to deliver them to acidified lysosomes. In AM from patients with tuberculosis, the bacilli were located in vacuoles that failed to accumulate endocytosed material and were not acidified. We concluded that AM from patients with tuberculosis and HIV infections were competent to endocytose and phagocytose material and to deliver the material to functional, acidified lysosomes. M. tuberculosis residing in these AM arrests the progression of their phagosomes, which fail to fuse with acidified lysosomes. This confirms, for the first time in humans with tuberculosis and HIV, the conclusions from previous animal and in vitro studies.—Authors’ Abstract


Using plasmid vaccination with DNA encoding the putative phosphate transport receptor PstS-3 from Mycobacterium tuberculosis and 36 overlapping 20-mer peptides spanning the entire PstS-3 sequence, we determined the immunodominant Th1-type CD4(+) T cell epitopes in C57BL/10 mice, as measured by spleen cell IL-2 and IFN-gamma production. Furthermore, a potent IFN-gamma-inducing, D(b)-restricted CD8(+) epitope was identified using MHC class I mutant B6.C-H-2(bm12) mice could be overcome by DNA vaccination. IFN-gamma-producing CD8(+) T cells could also be detected against the D(b)-restricted epitope in H-2(p) haplotype mice. These results highlight the potential of DNA vaccination for the induction and characterization of CD4(+) and particularly CD8(+) T cell responses against mycobacterial Ags.—Authors’ Abstract


The mobile insertion sequence, IS6110, is an important marker in tracking of Mycobacterium tuberculosis strains. Here, we demonstrate that IS6110 can upregulate downstream genes through an outward-directed promoter in its 3’ end, thus adding to the significance of this element. Promoter activity was orientation dependent and was localized within a 110 bp fragment adjacent to the right terminal inverted repeat. Transcripts from this promoter, named OP6110, begin approximately 85 bp upstream of the 3’ end of IS6110. Use of green fluorescent protein (GFP) expression constructs showed that OP6110 was upregulated in M. tuberculosis during growth in human monocytes and in late growth phases in broth. Analysis of natural insertion sites in M. tuberculosis showed that IS6110 upregulated expression of several downstream genes during growth.
in human monocytes, including Rv2280 in H37Rv and the PE-PGRS gene, Rv1468c, in the clinical strain 210, which is a member of the Beijing family. Transcription between IS6110 and downstream genes was confirmed by reverse transcription polymerase chain reaction. The ability to activate genes during infection suggests that IS6110 has the potential to influence growth characteristics of different strains, and indicates another mechanism by which IS6110 can impact \textit{M. tuberculosis} evolution.—Authors’ Abstract


\textit{Mycobacterium tuberculosis} possesses agonists for several Toll-like receptors (TLRs), yet mice with single TLR deletions are resistant to acute tuberculosis. MyD88(−/−) mice were used to examine whether TLRs play any role in protection against aerogenic \textit{M. tuberculosis} H37Rv infection. MyD88(−/−) mice failed to control mycobacterial replication and rapidly succumbed. Moreover, expressions of interleukin 12, tumor necrosis factor alpha, gamma interferon, and nitric oxide synthase 2 were markedly decreased in the knockout animals. These results argue that resistance to \textit{M. tuberculosis} must depend on MyD88-dependent signals mediated by an as-yet-undetermined TLR or a combination of TLRs.—Authors’ Abstract


The recently discovered RD1 locus encodes proteins that are actively secreted by pathogenic mycobacteria, including \textit{Mycobacterium tuberculosis}. Since they are missing in non-tuberculous mycobacteria, these proteins are promising not only as candidates for vaccination and diagnostic tests, but also in understanding mycobacterial evasion of protective immunity in susceptible individuals. Here we analyze the possible role of \textit{M. tuberculosis} secretory proteins in immunity against tuberculosis, with emphasis on their immunomodulatory action and the potential involvement in mycobacterial subversion of the host immune defense.—Authors’ Abstract

Microbiology


Mycolic acids represent a major component of the unique cell wall of mycobacteria. Mycolic acid biosynthesis is inhibited by isoniazid, a key frontline antitubercular drug that is inactivated by mycobacterial and human arylamine N-acetyltransferase (NAT). We show that an in-frame deletion of \textit{Mycobacterium bovis} BCG nat results in delayed entry into log phase, altered morphology, altered cell wall lipid composition, and increased intracellular killing by macrophages. In particular, deletion of nat perturbs biosynthesis of mycolic acids and their derivatives and increases susceptibility of \textit{M. bovis} BCG to antibiotics that permeate the cell wall. Phenotypic traits are fully complemented by introduction of \textit{Mycobacterium tuberculosis} nat. We infer from our findings that NAT is critical to normal mycolic acid synthesis and hence other derivative cell wall components and represents a novel target for antituberculosis therapy. In addition, this is the first report of an endogenous role for NAT in mycobacteria.—Authors’ Abstract

Mycobacterium tuberculosis persistence in human populations relies on its ability to inhibit phagosomal maturation. M. tuberculosis resides in a pathogen-friendly phagosome escaping lysosomal bactericidal mechanisms and efficient antigen presentation in the host phagocytic cell. M. tuberculosis phagosome maturation arrest includes the action of mycobacterial lipid products, which mimic mammalian phosphatidylinositols, targeting host cell membrane trafficking processes. These products interfere with membrane trafficking and organelle biogenesis processes initiated by Ca(2+) fluxes, and ending with host cell Rab GTP-binding proteins and their effectors. The block includes phosphatidylinositol 3-kinase and membrane tethering molecules that prepare phagosomes for fusion with other organelles. Understanding these processes could provide new targets for pharmacological intervention in tuberculosis.—Authors’ Abstract


Methionine sulfoxide reductase A (MsrA) is an antioxidant repair enzyme which reduces oxidized methionine to methionine. Since oxidation of methionine in proteins impairs their function, an absence of MsrA leads to abnormalities in different organisms, including alterations in the adherence patterns and in vivo survival of certain pathogenic bacteria. To understand the role of MsrA in intracellular survival of bacteria, we disrupted the gene encoding MsrA in Mycobacterium smegmatis through homologous recombination. The msrA mutant strain of M. smegmatis exhibited significantly reduced intracellular survival in murine J774A.1 macrophages compared to the survival of its wild-type counterpart. Furthermore, immunofluorescence and immunoblotting of phagosomes containing M. smegmatis strains revealed that the phagosomes with the msrA mutant strain acquired both p67(phox) of phagocyte NADPH oxidase and inducible nitric oxide synthase much earlier than the phagosomes with the wild-type strain. In addition, the msrA mutant strain of M. smegmatis was observed to be more sensitive to hydroperoxides than the wild-type strain was in vitro. These results suggest that MsrA plays an important role in both extracellular and intracellular survival of M. smegmatis.—Authors’ Abstract


The recent determination of the complete genome sequence of Corynebacterium diphtheriae, the aetiological agent of diphtheria, has allowed a detailed comparison of its physiology with that of its closest sequenced pathogenic relative Mycobacterium tuberculosis. Of major importance to the pathogenicity and resilience of the latter is its particularly complex cell envelope. The corynebacteria share many of the features of this extraordinary structure although to a lesser level of complexity. The cell envelope of M. tuberculosis has provided the molecular targets for several of the major antitubercular drugs. Given a backdrop of emerging multi-drug resistant strains of the organism (MDR-TB) and its continuing global threat to human health, the search for novel anti-tubercular agents is of paramount importance. The unique structure of this cell wall and the importance of its integrity to the viability of the organism suggest that the search for novel drug targets within the array of enzymes responsible for its construction may prove fruitful. Although the application of modern bioinformatics techniques to the “mining” of the M. tuberculosis genome has already increased our knowledge of the biosynthesis and assembly of the mycobac-
We have developed a colorimetric microtiter plate hybridization assay in order to simplify detection of *Mycobacterium leprae* in clinical specimens. This system detects the products amplified by a sensitive RT-PCR assay targeting a species-specific sequence of the bacterial 16S rRNA. The assay detected as few as 10 bacilli isolated from infected nude mouse lymph nodes or human skin biopsies. Sensitivity for diagnosis of clinical specimens was assessed for 58 tissue biopsies from untreated leprosy patients. The assay detected *M. leprae* RT-PCR products in 100% of biopsies from patients with multibacillary disease and 80% of biopsies from patients with paucibacillary disease, for an overall sensitivity of 91.3%. The test was highly specific as no RT-PCR products were amplified from skin biopsies of normal individuals or patients with skin diseases other than leprosy. The colorimetric assay is faster, more sensitive, and simplifies detection of RT-PCR products compared to Southern blot analysis. It may be useful for diagnosis of difficult cases of leprosy, and, since RNA is rapidly degraded after cell death, it may be appropriate for assessing response to therapy and for distinguishing relapse from reaction.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 418.


Acquisition of genetic information through horizontal gene transfer (HGT) is an important evolutionary process by which microorganisms gain novel phenotypic characteristics. In pathogenic bacteria, for example, it facilitates maintenance and enhancement of virulence and spread of drug resistance. In
the genus Mycobacterium, to which several primary human pathogens belong, HGT has not been clearly demonstrated. The few existing reports suggesting this process are based on circumstantial evidence of similarity of sequences found in distantly related species. Here, direct evidence of HGT between strains of Mycobacterium avium representing two different serotypes is presented. Conflicting evolutionary histories of genes encoding elements of the glycopeptidolipid (GPL) biosynthesis pathway led to an analysis of the GPL cluster genomic sequences from four Mycobacterium avium strains. The sequence of M. avium strain 2151 appeared to be a mosaic consisting of three regions having alternating identities to either M. avium strains 724 or 104. Maximum-likelihood estimation of two breakpoints allowed a approximately 4100 bp region horizontally transferred into the strain 2151 genome to be pinpointed with confidence. The maintenance of sequence continuity at both breakpoints and the lack of insertional elements at these sites strongly suggest that the integration of foreign DNA occurred by homologous recombination. To our knowledge, this is the first report to demonstrate naturally occurring homologous recombination in Mycobacterium. This previously undiscovered mechanism of genetic exchange may have major implications for the understanding of Mycobacterium pathogenesis.—Authors’ Abstract


Alternative sigma factors are key global regulators that coordinate bacterial responses to environmental changes necessary for adaptation and survival. In turn these sigma factors are controlled by regulators such as anti-sigma and anti-anti-sigma factors. In this report, using a cDNA-total RNA subtractive hybridization strategy that we have developed previously, we identified increased transcription of a potential sigma factor regulatory gene, Rv1364c, in Mycobacterium bovis BCG upon phagocytosis by macrophages and this was confirmed by Northern blot analysis. Primer extension analysis revealed the use of alternative promoters, P1 and P2, and that the increased expression inside macrophages coincided with promoter switching from P2 to P1. Rv1364c (653 amino acids), originally annotated as RsbU, contains structural domains homologous to the PAS redox sensor, the protein phosphatases anti-anti-sigma factor RsbU/SpoIIE, the protein kinase anti-sigma factor RsbW/SpoIIAB and the anti-anti-sigma factor RsbV/SpoIIAA found in other bacteria. These findings have important implications for understanding coordination of the expression of sigma factors under intra-macrophage conditions. Other potentially differentially expressed genes,
including genes for fatty acid metabolism, membrane transportors, heat shock proteins, potential sigma factors and energy metabolic pathways are also listed and their biological significance discussed.—Authors’ Abstract


DPPD is a *Mycobacterium tuberculosis* recombinant antigen that elicits specific delayed type hypersensitivity reactions similar in size and morphological aspects to that elicited by purified protein derivative, in both guinea pigs and humans infected with *M. tuberculosis*. In addition, earlier clinical studies with DPPD suggested that this molecule could improve the specificity of the tuberculin skin test, which is used as an important aid for the diagnosis of tuberculosis. However, these studies could only be performed with DPPD engineered as a fusion molecule with another *Mycobacterium* spp. protein because no expression of DPPD could be achieved as a single molecule or as a conventional fusion protein in any commercial system. Although recombinant fusion proteins are in general suitable for several biological studies, they are by definition not ideal for studies involving highly purified and defined polypeptide sequences. Here, we report two alternative approaches for the expression of immunologically reactive recombinant genuine DPPD. The first approach used the rapidly growing, non-pathogenic *Mycobacterium smegmatis* as host cells transformed with the pSMT3 plasmid vector containing the full-length DPPD gene. The second approach used Escherichia coli transformed with the pET-17b plasmid vector containing the DPPD gene engineered in a three-copy fusion manner in tandem with itself. Though at low levels, expression and purification of immunologically reactive DPPD in *M. smegmatis* could be achieved. More abundant expression and purification of DPPD as a homo-trimer molecule was achieved in *E. coli* (≥2 mg/L of bacterial broth cultures). Interestingly, expression could only be achieved in host cells transformed with the DPPD gene containing its leader peptide. However, the expressed proteins lacked the leader sequence, which indicates that processing of the *M. tuberculosis* DPPD gene was accurately achieved and necessary in both *M. smegmatis* and *E. coli*. More importantly, the delayed type hypersensitivity reactions elicited by purified molecules in guinea pigs infected with *M. tuberculosis* were indistinguishable from that elicited by purified protein derivative. Because the DPPD gene is present only in the tuberculosis-complex organisms of the *Mycobacterium* genus, these highly purified molecules should be helpful in identifying individuals sensitized with tubercle bacilli.—Authors’ Abstract


Porins mediate the diffusion of hydrophilic solutes across the outer membrane of mycobacteria, but the efficiency of this pathway is very low compared to Gram-negative bacteria. To examine the importance of porins in slow-growing mycobacteria, the major porin MspA of *Mycobacterium smegmatis* was expressed in *Mycobacterium tuberculosis* and *Mycobacterium bovis*. Approximately 20 and 35 MspA molecules per microm² cell wall were observed in *M. tuberculosis* and *M. bovis* BCG, respectively, by electron microscopy and quantitative immunoblot experiments. Surface accessibility of MspA in *M. tuberculosis* was demonstrated by flow cytometry. Glucose uptake was twofold faster, indicating that the outer membrane permeability of *M. bovis* BCG to small and hydrophilic solutes was increased by MspA. This significantly accelerated the growth of *M. bovis* BCG, identifying very slow nutrient uptake as one of the determinants of slow growth in mycobacteria. The suscep-
tibility of both M. bovis BCG and M. tuberculosi
to zwitterionic beta-lactam antibiotics was substantially enhanced by MspA, decreasing the minimal inhibitory concentration up to 16-fold. Furthermore, M. tuberculosis became significantly more susceptible to isoniazid, ethambutol and streptomycin. Fluorescence with the nucleic acid binding dye SYTO 9 was 10-fold increased upon expression of mspA. These results indicated that MspA not only enhanced the efficiency of the porin pathway, but also that of pathways mediating access to large and/or hydrophobic agents. This study provides the first experimental evidence that porins are important for drug susceptibility of M. tuberculosis.—Authors’ Abstract


Recombination is a ubiquitous genetic process which results in the exchange of DNA between two substrates. Homologous recombination occurs between DNA species with identical sequence whereas illegitimate recombination can occur between DNA with very little or no homology. Site-specific recombination is often used by temperate phages to stably integrate into bacterial chromosomes. Characterisation of the mechanisms of recombination in mycobacteria has mainly focussed on RecA-dependent homologous recombination and phage-directed site-specific recombination. In contrast the high frequency of illegitimate recombination in slow-growing mycobacteria has not been explained. The role of DNA repair in dormancy and infection have not yet been fully established, but early work suggests that RecA-mediated pathways are not required for virulence. All three recombination mechanisms have been utilized in developing genetic techniques for the analysis of the biology and pathogenesis of mycobacteria. A recently developed method for studying essential genes will generate further insights into the biology of these important organisms.—Authors’ Abstract


Mannosyltransferases play a crucial role in mycobacterial cell-wall biosynthesis and are potential new drug targets for the treatment of tuberculosis. Herein, we describe the synthesis of alpha-(1→2)- and alpha-(1→6)-linked manno-pyransyl disaccharides possessing a 5-azidonaphthlene-1-sulfonamidoethyl group as photoaffinity probes for active-site labeling studies of mannosyltransferases in Mycobacterium tuberculosis.—Authors’ Abstract

Sputum smear microscopy is the most efficient and rapid technique for detection of acid-fast bacilli (AFB). Fluorochrome method of staining is preferred for Mycobacteria in the overburdened laboratories as the fluorescing bacilli are more readily detected than the fuchsin stained bacilli in shorter period of time. A total of 300 sputum samples obtained from suspected cases of Tuberculosis were collected and were subjected to staining by rhodamine auramine at 37 degrees C and also at room temperature (conventional method). The smears were then blindly evaluated. Fifty-eight samples were positive by both methods and 5 were positive at 37 degrees C only. Staining at 37 degrees C increased the smear positivity by 8.6% over conventional staining at room temperature. No smears were positive only with staining at room temperature alone. Out of 58 smears positive by both methods, 25 had equal number of AFB in both smears, 22 had more AFB in smear stained at 37 degrees C and 11 had greater number of AFB in smears stained at room temperature. Our study, therefore, indicates that rhodamine auramine staining at 37 degrees C is superior to conventional auramine method at room temperature for detecting AFB in sputum smears.—Authors’ Abstract


Serine/threonine protein kinases (STPKs) represent a burgeoning concept in prokaryotic signaling and have been implicated in a range of control mechanisms. This paper describes the enzymatic and molecular characterization of PknH, a mycobacterial STPK. After cloning and expression as a Glutathione-S-transferase fusion protein in E. coli, PknH was found to phosphorylate itself and exogenous substrates like myelin basic protein and histone. The kinase activity of PknH was inhibited by the kinase inhibitors staurosponine and H-7. The results confirmed that PknH is a transmembrane protein and is restricted to members of the *Mycobacterium tuberculosis* complex. In addition, transcriptional analysis of pknH in *M. tuberculosis* under various stress conditions revealed that exposure to low pH and heat shock decreased the level of pknH transcription significantly. This is the first report describing differential expression of a mycobacterial kinase in response to stress conditions which can indicate its ability to regulate cellular events promoting bacterial adaptation to environmental change.—Authors’ Abstract


The recent publication of the genome sequence of *Mycobacterium bovis* showed >99.95% identity to *M. tuberculosis*. No genes unique to *M. bovis* were found. Instead numerous single-nucleotide polymorphisms (SNPs) were identified. This has led to the hypothesis that differential gene expression due to SNPs might explain the differences between the human and bovine tubercle bacilli. One phenotypic distinction between *M. tuberculosis* and *M. bovis* is nitrate reduction, which not only is an essential diagnostic tool but also contributes to mycobacterial pathogenesis. We previously showed that narGHJI encodes a nitrate reductase in both *M. tuberculosis* and *M. bovis* and that NarGHJI-mediated nitrate reductase activity was substantially higher in the human tubercle bacillus. In the present study we used a genetic approach to demonstrate that an SNP within the promoter of the nitrate reductase gene cluster narGHJI is responsible for the different nitrate reductase activity of *M. tuberculosis* and *M. bovis*. This is the first example of an SNP that leads to differential gene expression between the human and bovine tubercle bacilli.—Authors’ Abstract

This paper describes a method for isolation of deoxyribonucleic acid (DNA) from Ziehl-Neelsen stained sputum smears on glass slides; and isolated DNA was used for the IS6110 polymerase chain reaction (PCR)-based identification of *M. tuberculosis*. A total of 221 samples from newly diagnosed suspected tuberculosis cases were first examined by microscopic examination. For DNA extraction by silica-based filter, a home-made modified spin column gave the efficacy as did the nucleospin tissue reagent kit and therefore was selected for PCR template preparation. The extracted DNA was amplified by the IS6110 PCR using a primer pair that amplifies a 377-bp target, and the product was analyzed by agarose gel electrophoresis with confirmation by Southern blot hybridization. In comparison with culture, PCR with template prepared by the silica based filter showed overall sensitivity and specificity of 91.7 and 100 per cent, respectively. This study used the over one year and less than one year slides samples to study the effect of storage time. In the more than one year storage group, PCR assay gave a sensitivity and specificity of 83.3 and 100 per cent, respectively. In conclusion, the applicability of the PCR directly to DNA extracted from Ziehl-Neelsen stained smears could become a valuable alternative approach for rapid identification of *M. tuberculosis*, and could be used to evaluate quality of the control of local laboratories in tuberculosis (TB) screening and solve the problem of specimen transportation. In addition, the method could be used in retrospective studies involving a wide range of PCR-based analyses, such as detection of rifampicin resistant gene in multidrug-resistant tuberculosis (MDR-TB) study.—Authors’ Abstract


The need for molecular tools for the differentiation of isolates of *Mycobacterium leprae*, the organism that causes leprosy, is urgent in view of the continuing high levels of new case detection, despite years of aggressive chemotherapy and the consequent reduction in the prevalence of leprosy. The slow onset of leprosy and the reliance on physical examination for detection of disease have restricted the epidemiological tracking necessary to understand and control transmission. Two genetic loci in several isolates of *M. leprae* have previously been demonstrated to contain variable-number tandem repeats (VNTRs). On the basis of these reports and the availability of the full genome sequence, multiple-locus VNTR analysis for strain typing has been undertaken. A panel of 11 short tandem repeat (STR) loci with repeat units of 1, 2, 3, 6, 12, 18, 21, and 27 bp from four clinical isolates of *M. leprae* propagated in armadillo hosts were screened by PCR. Fragment length polymorphisms were detected at 9 of the 11 loci by agarose gel electrophoresis. Sequencing of representative DNA products confirmed the presence of VNTRs between isolates. The application of nine new polymorphic STRs in conjunction with automated methods for electrophoresis and size determination allows greater discrimination between isolates of *M. leprae* and enhances the potential of this technique to track the transmission of leprosy.—Authors’ Abstract


The deciphering of the genomic sequence of *Mycobacterium leprae* has made possible
to predict the possible lipoproteins. The consensus sequence at the N-terminal region of the protein, including the cysteine residue to which the lipid moiety gets attached, provides a clue to the search. As such, more than 20 putative lipoproteins have been identified from *Mycobacterium leprae* genomic sequence. Lipoprotein LpK (Accession no. ML0603) which encodes for 371 amino acid precursor protein, was identified. Expression of the protein, in Escherichia coli revealed a 33 kD protein, and metabolic labeling experiments proved that the protein was lipidated. The purified lipoprotein was found to induce production of IL-12 in human peripheral blood monocytes which may imply that *M. leprae* LpK is involved in protective immunity against leprosy. Pursuit of such lipoproteins may reveal insights into the pathogenesis of the disease.—Authors’ Abstract


We developed a live, fully attenuated *Mycobacterium tuberculosis* vaccine candidate strain with two independent attenuating auxotrophic mutations in leucine and pantothenate biosynthesis. The deltaleuD deltapanCD double auxotroph is fully attenuated in the SCID mouse model and highly immunogenic and protective in the extremely sensitive guinea pig tuberculosis model, reducing both bacterial burden and disease pathology.—Authors’ Abstract


It has not been possible to distinguish different strains of *Mycobacterium leprae* according to their genetic sequence. However, the genome contains several variable-number tandem repeats (VNTR), which have been used effectively in strain typing of other bacteria. To determine their suitability for differentiating *M. leprae*, we developed PCR systems to amplify 5 different VNTR loci and examined a battery of 12 *M. leprae* strains derived from patients in different regions of the United States, Brazil, Mexico, and the Philippines, as well as from wild armadillos and a sooty mangabey monkey. We found diversity at four VNTR (D = 0.74), but one system (C(16)G(8)) failed to yield reproducible results. Alleles for the GAA VNTR varied in length from 10 to 16 copies, those for AT(17) varied in length from 10 to 15 copies, those for GTA varied in length from 9 to 12 copies, and those for TA(18) varied in length from 13 to 20 copies. Relatively little variation was seen with interspecies transfer of bacilli or during short-term passage of strains in nude mice or armadillos. The TA(18) locus was more polymorphic than other VNTR, and genotypic variation was more common after long-term expansion in armadillos. Most strain genotypes remained fairly stable in passage, but strain Thai-53 showed remarkable variability. Statistical cluster analysis segregated strains and passage samples appropriately but did not reveal any particular genotype associable with different regions or hosts of origin. VNTR polymorphisms can be used effectively to discriminate *M. leprae* strains. Inclusion of additional loci and other elements will likely lead to a robust typing system that can be used in community-based epidemiological studies and select clinical applications.—Authors’ Abstract


Phenolic glycolipid-I (PGL-I), a *Mycobacterium leprae*-specific antigen, has been widely used for the serodiagnosis of leprosy and has been implicated in the pathogenesis of leprosy. In an effort to produce an al-
ternate antigen of PGL-I, the mimotope peptides of PGL-I, W(T/R)LGPY(V/M), were obtained using a monoclonal antibody, III603.8, specific to PGL-I by a phage library. The biotin-labeled predominant mimotope peptide of PGLP1, WTLGPYV, bound to III603.8 in a dose-dependent manner in an immunoassay. However, PGLP1 did not bind to anti-PGL-I antibodies in the serum samples from leprosy patients that were reactive to PGL-I. Although the mimotope peptide of WTLGPYV was not effective as an alternate antigen of PGL-I for the serodiagnosis of leprosy, it would be of interest to know how the mimotope peptides mimic the role of PGL-I antigen in the pathogenesis of M. leprae infection.—Authors’ Abstract

Microbiology, Tuberculosis


P27 lipoprotein was previously described as an antigen in the Mycobacterium tuberculosis complex, encoded by the lprG gene, also named Rv1411 in the TuberculList (http://genolist.pasteur.fr/TuberculList) gene bank. It forms an operon with Rv1410 that encodes for an efflux pump, P55. A mutant of the H37Rv strain of M. tuberculosis not producing P27 (strain DeltaP27) was obtained by two-step mutagenesis using the counterselectable marker sacB and a thermosensitive origin of replication in the shuttle plasmid pPR27. By RT-PCR, we observed no lprG or Rv1410 mRNA in the DeltaP27 mutant strain compared with the wild type and complemented strains. Western blot experiments using anti-P27 polyclonal sera showed that the P27 protein was present both in the parental and in a complemented strain, in which the entire lprG-Rv1410 operon was reintroduced, but absent in the mutant strain. The three strains showed similar growth kinetics and characteristics in culture broth. To study the effect of the lprG mutation on M. tuberculosis virulence, BALB/c mice were inoculated to determine bacterial loads in spleens. At days 15 and 35 after infection, decreases of 1.5 and 2.5 logs in the bacterial load were found, respectively, in animals inoculated with the DeltaP27 mutant strain or with the wild type. This attenuation was reverted in the complemented strain. These results demonstrated that lprG gene is required for growth of M. tuberculosis in immunocompetent mice. The reversion of attenuation in the complemented strain indicates that the attenuated phenotype resulted from disruption of the lprG-Rv1410 operon.—Authors’ Abstract


We have identified an omega,E,E-farnesyl diphosphate (omega,E,E-FPP) synthase, encoded by the open reading frame Rv3398c, from Mycobacterium tuberculosis that is unique among reported FPP synthases in that it does not contain the type I (eukaryotic) or the type II (eubacterial) omega,E,E-FPP synthase signature motif. Instead, it has a structural motif similar to that of the type I geranylgeranyl diphosphate synthase found in Archaea. Thus, the enzyme represents a novel class of omega,E,E-FPP synthase. Rv3398c was cloned from the M. tuberculosis H37Rv genome and expressed in Mycobacterium smegmatis using a new mycobacterial expression vector (pVV2) that encodes an in-frame N-terminal affinity tag fusion with the protein of interest. The fusion protein was well expressed and could be purified to near homogeneity, allowing facile kinetic analysis of recombinant Rv3398c. Of the potential allylic substrates tested, including dimethylallyl diphosphate, only geranyl diphosphate served as an acceptor for isopentenyl diphosphate.
The enzyme has an absolute requirement for divalent cation and has a $K(m)$ of 43 microM for isopentenyl diphosphate and 9.8 microM for geranyl diphosphate and is reported to be essential for the viability of $M. tuberculosis$.—Authors’ Abstract


Mycobacterium tuberculosis is an important human pathogen in virtually every part of the world. Here we investigate whether distinct strains of $M. tuberculosis$ infect different human populations and whether associations between host and pathogen populations are stable despite global traffic and the convergence of diverse strains of the pathogen in cosmopolitan urban centers. The recent global movement and transmission history of 100 $M. tuberculosis$ isolates was inferred from a molecular epidemiologic study of tuberculosis that spans 12 years. Genetic relationships among these isolates were deduced from the distribution of large genomic deletions, which were identified by DNA microarray and confirmed by PCR and sequence analysis. Phylogenetic analysis of these deletions indicates that they are unique event polymorphisms and that horizontal gene transfer is extremely rare in $M. tuberculosis$. In conjunction with the epidemiological data, phylogenies reveal three large phylogeographic regions. A host’s region of origin is predictive of the strain of tuberculosis he or she carries, and this association remains strong even when transmission takes place in a cosmopolitan urban center outside of the region of origin. Approximate dating of the time since divergence of East Asian and Philippine clades of $M. tuberculosis$ suggests that these lineages diverged centuries ago. Thus, associations between host and pathogen populations appear to be highly stable.—Authors’ Abstract


In the present study, we demonstrate that, in analogy with the genes encoding ESAT-6 and CFP-10, the genes rv0287 and rv0288 from the ESAT-6 gene family are cotranscribed. Using Western-Western blotting and protein-print overlay methodologies, we demonstrate that ESAT-6 and CFP-10, as well as the protein pair Rv0288/Rv0287, interact pairwise in a highly specific way. Most notably, the ESAT-6 proteins interact directly with Rv3873, a possible cell envelope component of the ESAT-6 secretion pathway.—Authors’ Abstract


We have reported that Mycobacterium tuberculosis residing within the phagosomes of human monocyte-derived macrophages (MDM) can acquire Fe from extracellular transferrin (TF) and sources within the MDM. In the lung, Fe is also bound to lactoferrin (LF) and low-molecular-weight


Erp (exported repetitive protein) is a member of a mycobacterium-specific family of extracellular proteins. A hydrophobic region that is localized at the C-terminal domain and that represents a quarter of the protein is highly conserved across species. Here we show that this hydrophobic region is not essential for restoring the virulence and tissue damage of an erp::aph mutant strain of $M. tuberculosis$ as assessed by bacterial counts and lung histology analysis in a mouse model of tuberculosis.—Authors’ Abstract
chelates. We therefore investigated the ability of intraphagosomal M. tuberculosis to acquire Fe from these sources. M. tuberculosis acquired 30-fold and 3-fold more Fe from LF and citrate, respectively, compared to TF, in spite of similar MDM-associated Fe. M. tuberculosis infection decreased MDM-associated Fe relative to uninfected MDM as follows: TF (38.7%), citrate (21.1%), and LF (15.3%). M. tuberculosis Fe acquisition from extracellular chelates (exogenous source) and from endogenous MDM Fe initially acquired from the three chelates (endogenous source) was compared. M. tuberculosis Fe acquisition was similar from exogenous and endogenous sources supplied as Fe-TF. In contrast, there was much greater intracellular M. tuberculosis Fe uptake from LF and citrate from the exogenous than endogenous source. Gamma interferon (IFN-gamma) reduced MDM Fe uptake from each chelate by approximately 50% and augmented the M. tuberculosis-induced decrease in MDM Fe uptake from exogenous TF, but not from LF or citrate. IFN-gamma minimally decreased intracellular M. tuberculosis Fe acquisition from exogenous Fe-TF but significantly increased Fe uptake from LF and citrate. Intraphagosomal M. tuberculosis Fe acquisition from both exogenous and endogenous MDM sources, and the effect of IFN-gamma on this process, is influenced by the nature of the extracellular Fe chelate. M. tuberculosis has developed efficient mechanisms of acquiring Fe from a variety of Fe chelates that it likely encounters within the human lung.—Authors’ Abstract


The gene for histone-like protein (hupB [Rv2986c]) of Mycobacterium tuberculosis has been identified as a singular target which allows differentiation of two closely related mycobacterial species, namely, M. tuberculosis and M. bovis of the MTB complex, by a PCR assay. The N and S primer-generated PCR amplicons differed in M. tuberculosis and M. bovis; these amplicons were determined to be 645 and 618 bp, respectively. This difference was localized to the C-terminal part of the gene by using primers M and S. The C-terminal PCR amplicons of M. tuberculosis and M. bovis were determined to be 318 and 291 bp, respectively. The differences in the C-terminal portion of the gene were confirmed by re-
striction fragment length polymorphism analysis and sequencing. Sequence analysis indicated that in *M. bovis* there was a deletion of 27 bp (9 amino acids) in frame after codon 128 in the C-terminal part of the hupB gene. In the present study 104 mycobacterial strains and 11 nonmycobacterial species were analyzed for hupB gene sequences. Of the 104 mycobacterial strains included, 62 belonged to the MTB complex and 42 were non-MTB complex strains and species. Neither the hupB gene-specific primers (N and S) nor the C-terminal primers (M and S) amplify DNA from any other mycobacteria, making the assay suitable for distinguishing members of the MTB complex from other mycobacterial species, as well as for differentiating between members of the MTB complex, namely, *M. tuberculosis* and *M. bovis*.—Authors’ Abstract


Arrest of the multiplication of *Mycobacterium tuberculosis* caused by expression of adaptive immunity in mouse lung was accompanied by a 10- to 20-fold decrease in levels of mRNAs encoding the secreted Ag85 complex and 38-kDa lipoprotein, esat-6 mRNA levels were high throughout infection. The data imply that multiplying and nonreplicating tubercle bacilli have different antigen compositions.—Authors’ Abstract


BACKGROUND: One mechanism proposed for drug resistance in *Mycobacterium tuberculosis* (MTB) is by efflux of the drugs by membrane located pumps. We report a novel and definite association between drug resistance and transcription levels of a tap-like pump (Rv1258c) in a multi-drug resistant MTB patient isolate (ICC154) which possesses a unique genotypic signature. MATERIALS AND METHODS: The isolate ICC154 was tested for drug sensitivity. Over-expression of Rv1258c as a function of drug pressure was analyzed by RT-PCR and the strain was typed using fluorescent amplified fragment length polymorphism (FAFLP). RESULT: In the presence of rifampicin and ofloxacin, this isolate shows increased transcription of the gene Rv1258c. Genotypic fingerprinting revealed the presence of unique FAFLP markers. CONCLUSION: A clear association between drug resistance and overexpression of an efflux protein is evident from our studies. The presence of specific markers has implications in rapid identification of MDR clinical isolates and consequent disease management.—Authors’ Abstract


The ability of *Mycobacterium tuberculosis* to grow in macrophages is central to its pathogenicity. We found previously that the widespread 210 strain of *M. tuberculosis* grew more rapidly than other strains in human macrophages. Because principal sigma factors influence virulence in some bacteria, we analysed mRNA expression of the principal sigma factor, sigA, in *M. tuberculosis* isolates during growth in human macrophages. Isolates of the 210 strain had higher sigA mRNA levels and higher intracellular growth rates, compared with other clinical strains and the laboratory strain H37Rv. SigA was also upregulated in the 210 isolate TB294 during growth in macrophages, compared with growth in broth. In contrast, H37Rv sigA mRNA levels did not change under these conditions. Overexpression of sigA enhanced growth of recombinant *M. tuberculosis* in macrophages and in lungs of mice after aerosol infection, whereas recombinant strains expressing antisense transcripts to sigA showed decreased
growth in both models. In the presence of superoxide, sense sigA transformants showed greater resistance than vector controls, and the antisense sigA transformant did not grow. We conclude that *M. tuberculosis* sigA modulates the expression of genes that contribute to virulence, enhancing growth in human macrophages and during the early phases of pulmonary infection *in vivo*. This effect may be mediated in part by increased resistance to reactive oxygen intermediates.—Authors’ Abstract

**Experimental Infections**


The hygiene hypothesis proposes that common, harmless microorganisms, present throughout our evolutionary history, have helped to develop immunoregulatory mechanisms that prevent inappropriate immune responses by the host. Using a mouse model of allergic pulmonary inflammation, we report that treatment with an ubiquitous saprophytic mycobacterium, *Mycobacterium vaccae*, significantly reduces allergic inflammation by decreasing type 2 responses such as eosinophilia and IL-4 expression. Rather than observing an increase in type-1 cytokine expression, we found elevated production of IL-10 in the lungs suggesting a role for regulatory T cells. Since induction of these cells may be dependent on APC, we investigated the effects of *M. vaccae* treatment on pulmonary CD11c+ cells. Increased levels of IL-10, TGF-beta and IFN-alpha mRNA were detected in CD11c+ cells from *M. vaccae*-treated allergic mice. We propose that *M. vaccae*-induced CD11c+ cells have a potential regulatory role at the site of inflammation through their secretion of immunomodulatory cytokines.—Authors’ Abstract


Thalidomide is a selective inhibitor of tumor necrosis factor-alpha (TNF-alpha), a cytokine involved in mycobacterial death mechanisms. We investigated the role of this drug in the functional activity of alveolar macrophages in the presence of infection induced by intranasal inoculation of *Mycobacterium avium* in thalidomide-treated and untreated adult Swiss mice. Sixty animals were inoculated with $5 \times 10^6$ *M. avium* by the respiratory route. Thirty animals received daily thalidomide (30 mg/kg mouse) and 30 received water by gavage up to sacrifice. Ten non-inoculated mice were used as a control group. Lots of animals from each group were evaluated until 6 weeks after inoculation. Infection resulted in an increased total number of inflammatory cells as well as increased activity of pulmonary macrophages. Histologically, intranasal inoculation of bacilli resulted in small mononuclear infiltrates located at the periphery of the organ. Culture of lung fragments revealed the presence of bacilli only at the beginning and at the end of the experimental period. Thalidomide administration did not affect the microbiological or histological features of the infection. Thalidomide-treated and untreated animals showed the same amount of *M. avium* colonies 3 weeks after infection. Although it did not affect bacillary clearance, thalidomide administration resulted in a decreased percent of spread cells and release of hydrogen peroxide, suggesting that factors other than TNF-alpha play a role in the killing of mycobacteria by alveolar macrophages. Thalidomide administration also reduced the number of spread cells among resident macrophages, suggesting a direct effect of the drug on this phenomenon.—Authors’ Abstract

BCG vaccines are a family of closely related daughter strains of an attenuated isolate of Mycobacterium bovis derived by in vitro passage from 1908 to 1921. During subsequent laboratory propagation of the vaccine strain until its lyophilization in 1961, BCG Pasteur underwent at least seven further genomic mutations. The impact of these mutations on the properties of the vaccine is currently unknown. One mutation, a glycine-to-aspartic acid substitution in the mmaA3 gene, occurred between 1927 and 1931 and impairs methoxymycolic acid synthesis in BCG strains obtained from the Pasteur Institute after this period. Mycolic acids of the cell wall are classified into three functional groups (alpha-, methoxy-, and keto-mycolic acids), and together these lipids form a highly specialized permeability barrier around the bacterium. To explore the impact of methoxymycolic acid production by BCG strains, we complemented the functional gene of mmaA3 into BCG Denmark and tested a number of in vitro and in vivo phenotypes. Surprisingly, restoration of methoxymycolic acids alone had no effect on cell wall permeability, resistance to antibiotics, or growth in cultured macrophages and C57BL/6 mice. Our results demonstrate that the loss of methoxy-mycolic acid production did not apparently affect the virulence of BCG strains.—Authors’ Abstract


The only vaccine currently available for the prevention of tuberculosis in man is a live attenuated vaccine, bacille Calmette-Guerin (BCG), derived from Mycobacterium bovis. Concerns over the lack of the universal efficacy and safety of BCG have resulted in efforts to develop a new generation of TB vaccines. Historically, killed whole-cell preparations of mycobacteria have been ineffective vaccines. We revisited the potential of killed whole-cell vaccines by comparing their efficacy with live BCG Pasteur in a guinea pig challenge model. BCG Pasteur was inactivated with a low concentration of formalin and showed to be non-viable in culture or severe combined immunodeficient mice. Formalin-inactivated BCG was mixed with non-phospholipid liposome adjuvants (Novasomes) and administered to guinea pigs as a single subcutaneous inoculation. All formulations were well tolerated and one conferred a significant survival advan-


In this study, we used an HLA-A2 transgenic mouse model to investigate the effects of pulmonary delivery of a new DNA plasmid encoding eight HLA-A*0201-restricted T-cell epitopes from Mycobacterium tuberculosis formulated in chitosan nanoparticles. It was shown that the chitosan-DNA formulation was able to induce the maturation of dendritic cells (DCs) while chitosan solution alone could not, indicating the DNA was released from the particles and able to stimulate DCs. Pulmonary administration of the DNA plasmid incorporated in chitosan nanoparticles was shown to induce increased levels of IFN-gamma secretion compared to pulmonary delivery of plasmid in solution or the more frequently used intramuscular immunization route. These results indicate that pulmonary delivery of DNA vaccines against tuberculosis may provide an advantageous delivery route compared to intramuscular immunization, and that increased immunogenicity can be achieved by delivery of this DNA encapsulated in chitosan nanoparticles.—Authors’ Abstract
tage against lethal aerogenic challenge with *M. bovis*.—Authors’ Abstract


Tuberculosis caused by infection with *Mycobacterium tuberculosis* or *Mycobacterium bovis* is a significant disease of man and animals. Whilst cellular immunity is the major immunological component required for protection against these organisms, recent reports have suggested that monoclonal antibodies can modify infection with *M. tuberculosis*. To test whether the same was true for *M. bovis* infection, we determined the effect of preincubation of *M. bovis* with a monoclonal antibody on subsequent intravenous infection of mice. Antibodies bound to the surface of *M. bovis* increased the survival time of mice infected with *M. bovis* and changed the morphology of granulomas and the distribution of acid-fast bacilli in the lung. These studies suggest that antibodies directed to the surface of virulent mycobacteria can modulate their virulence in vivo.—Authors’ Abstract


Efficient protein-based vaccine delivery systems are needed to achieve a persistent memory immune response capable of detecting and eliminating intracellular pathogens such as *Mycobacterium tuberculosis* (TB). We have developed a novel protein-microsphere formulation using the recently discovered TB antigen Mtb8.4. Immunization of mice with a single dose of this Mtb8.4-microsphere formulation resulted in both humoral and cellular responses against Mtb8.4. The Mtb8.4-specific CD8 T-cell responses following a single administration of Mtb8.4-microspheres exceeded that elicited by protein plus adjuvant following multiple immunizations. These results demonstrate the efficacy of a single dose protein-microsphere vaccine for the induction of strong cell-mediated and humoral immune responses against *M. tuberculosis* antigens.—Authors’ Abstract


Because of the availability of uniform genetic stocks and the ability to modulate stress levels, chickens were investigated as a host for the development of an antimycobacterial vaccine. The imposition and the timing of stress significantly influenced the outcome of *Mycobacterium avium* infection in chickens. Simple, whole cell or lysate vaccines and combinations of vaccine preparations were identified that led to high levels of protection. In addition, short-term stress at the time of vaccination significantly increased the protective efficacy of *M. avium* vaccine preparations. Post-infection vaccination of *M. avium*-infected chickens was also shown to significantly reduce the number of lesions and colony counts.—Authors’ Abstract


AIM: To investigate the effects of plasmid containing mouse IL-12 and human IL-18 genes on the humoral immune response of mice immunized by CFP10 gene of *Mycobacterium tuberculosis* (MTB) H(37)R\(^{v}\) strain. METHODS: Human IL-18 cDNA was amplified from RNA of PBMCs by RT-PCR and cloned into the pGEM-Teasy vector.
After sequencing it was subcloned into the sites of BamH I and EcoR I digestion of pcDNA3.1. BALB/c mice were injected intramuscularly by eukaryotic expression plasmid pcmIL12 and pcIL18, together with MTB CFP10 DNA vaccine, respectively. The same immunization repeated three times at intervals of two weeks. Mouse sera were collected at two weeks after each injection. The titer of anti-CFP10 antibody was detected by ELISA. RESULTS: IL-18 cDNA was amplified successfully from RNA of human PBMCs by RT-PCR and the result of sequencing was correct. The IL-18 gene was correctly inserted into the vector pcDNA3.1 by being confirmed with BamH I and EcoR I digestion analysis, positive plasmid was called pcIL18. After being immunized with pcCFP10 three times, the end point titer of anti-CFP10 was 1:4 000, while the titer obtained by being immunized with pcIL18 + pcCFP10 was 1:8 000, but yet, after being immunized with pcmIL12+pcCFP10, the end point titer of anti-CFP10 antibody was only 1:200. CONCLUSION: Combination of IL-18 gene with MTB CFP10 DNA vaccine can enhance the humoral immune responses to pcCFP10, whereas the immunization with IL-12 gene plus pcCFP10 made humoral immune response markedly descent. As for whether IL-18 gene plus MTB CFP10 DNA vaccine can induce markedly the cellular mediated immune response to CFP10 or not remains to be further investigated. — Authors’ Abstract


The current live attenuated vaccine against tuberculosis, BCG, poses a risk of disseminated infections in immunocompromised subjects. Therefore, in this study we compared the protective effect of a heat-killed bacille Calmette-Guerin (H-kBCG) vaccine given in a new adjuvant (Eurocine L3) with the protection provided by the conventional live attenuated BCG vaccine in mice (C57BL/6 and BALB/c) challenged with virulent Mycobacterium tuberculosis (strain Harlingen). The H-kBCG vaccine alone, in accordance with earlier studies, did not give any or only gave slight protection compared to sham-vaccinated controls. However, the same vaccine given with Eurocine L3 adjuvant, either formulated as a suspension or as an emulsion, afforded significant levels of protection. This protection was at least as good as that of the control live attenuated BCG vaccine. The Eurocine L3 adjuvant is approved for human use as a nasal vaccine adjuvant and a successful phase I trial with nasal immunization with diphtheria vaccine has recently been performed in Sweden. Here we show that, in mice, intranasal priming with H-kBCG in Eurocine L3 adjuvant followed by intranasal booster resulted in the same level of protection as subcutaneous priming followed by intranasal booster. All H-kBCG formulations in the Eurocine L3 adjuvant elicited mycobacterial antigen-specific serum IgG and IFN gamma responses. In general, among the different vaccine formulation(s) in the Eurocine L3 adjuvant those that produced a relatively high Th2 response, as measured by IgG1/IgG2a ratio and IFN gamma production in vitro, were the most protective. In conclusion, H-kBCG in Eurocine L3 adjuvant could represent a safe and a more stable alternative to the conventional live BCG vaccine. — Authors’ Abstract


Mycobacterium avium complex (MAC) infection is the most common disseminated bacterial infection in untreated patients with acquired immunodeficiency syndrome (AIDS). We investigated the potential role of monocyte chemoattractant protein-1 (MCP-1) in the pathogenesis of disseminated MAC, using the simian immunodeficiency virus (SIV)/macaque model of AIDS. Macaques were inoculated with SIV, followed by challenge
with a pathogenic AIDS isolate of *M. avium* 14 days later. After challenge with *M. avium*, marked increases in serum MCP-1 levels were detected in SIV-infected macaques, a finding that was duplicated in coinoculated bronchoalveolar macrophages. MCP-1 levels were significantly higher in SIV-infected macaques than in non-SIV-infected controls (327.1 vs. 151.5 pg/mL, respectively; *p* = 0.04), suggesting that up-regulation of MCP-1 contributes to the development of progressive mycobacterial disease. Similarly, morphometric analysis revealed increased expression of MCP-1 in hepatic microgranulomas from SIV-infected macaques. We conclude that the pronounced increases in MCP-1 levels demonstrated in tissue and serum samples after *M. avium* inoculation may play a role in the development of disseminated mycobacterial disease.—Authors’ Abstract


We reported previously that even though immunization with the recombinant mycobacterial 27-kDa lipoprotein (r27) induced a Th1-type response in mice, the vaccinated mice became more susceptible to challenge with *Mycobacterium tuberculosis*. In this study we show that r27 stimulates naive splenocytes to proliferate. Acylation of r27 was crucial for this effect, since a nonacylated mutant of r27, termed r27DeltaSP, failed to stimulate splenocytes either *in vitro* or *in vivo*. Depletion experiments indicated that only B cells were proliferating in a T-cell-independent manner. We also found that r27 is recognized by TLR2, which is involved in mitogenic stimulation. Interestingly, r27 but not r27DeltaSP induced high gamma interferon levels in splenocyte supernatants, whereas no significant interleukin-2 levels were detected. Since B-cell polyclonal activation might aggravate pathogen infection, we asked whether the antiprotective effect of the r27 lipoprotein is associated with its mitogenicity. We showed that, as in the case of r27, immunization of mice with the non-mitogenic r27DeltaSP lipoprotein resulted in increased *M. tuberculosis* multiplication. We conclude that the antiprotective effect of the r27 lipoprotein must be linked to properties of the polypeptide portion of the lipoprotein rather than to its lipid moiety and its mitogenicity.—Authors’ Abstract


*Mycobacterium leprae* (ML) GroES has been shown to induce strong T cell responses in tuberculoid as well as in exposed healthy contacts of leprosy patients, and therefore this antigen has been the focus of study as a potential vaccine candidate. Paradoxically, we have shown that ML GroES also induces extremely high titres of IgG1 antibody in leprosy patients across the disease spectrum, a response associated with disease progression. IgG1 antibodies in leprosy also show a negative association with interferon-gamma, a critical T cell cytokine responsible for macrophage activation and intracellular killing of mycobacteria. We therefore queried if antibody and T cell responses were being evoked by different epitopes in ML GroES proteins. To address the issue of epitope recognition in mycobacterial diseases, we have analysed 16 peptides (15-mer peptides) spanning the entire ML and *M. tuberculosis* GroES protein in leprosy (*N* = 19) and tuberculosis (*N* = 9) patients and healthy endemic controls (*N* = 8). Our analysis demonstrates clearly that the dominant peptides evoking T cell and IgG subclass antibodies were different. The target of both T and B cell responses were cross-reactive epitopes in all groups. Differences in disease and healthy states related to the strength (mean intensity) of the T cell and antibody response. IgG1 and IgG3 antibodies were associated with disseminated disease and IgG 2 and IgG4 with disease limitation. Such comprehensive immune
profiling of antigen-specific responses is critical to understanding the disease pathogenesis and also if these reagents are to be exploited for either diagnostic or vaccine purposes.—Authors’ Abstract


The proteins secreted by Mycobacterium tuberculosis are an important target for vaccine development. To identify the antigens from M. tuberculosis culture filtrate (CF) that strongly stimulate T-cells, the CF was fractionated by ion-exchange chromatography and then non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis with mini-whole gel elution. Each fraction was screened for its ability to induce interferon-gamma (IFN-gamma) production in peripheral blood mononuclear cells isolated from healthy tuberculin reactors. The protein bands that strongly induced IFN-gamma production were subjected to N-terminal sequencing. Two new proteins, a 17-kDa protein (Rv0164, MTSP17) and an 11-kDa protein (Rv3204, MTSP11) protein, were identified. The recombinant MTSP17 (rMTSP17) and rMTSP11 induced significant production of IFN-gamma and interleukin (IL)-12p40 in peripheral blood mononuclear cells isolated from healthy tuberculin reactors. The protein bands that strongly induced IFN-gamma production were subjected to N-terminal sequencing. Two new proteins, a 17-kDa protein (Rv0164, MTSP17) and an 11-kDa protein (Rv3204, MTSP11) protein, were identified. The recombinant MTSP17 (rMTSP17) and rMTSP11 induced significant production of IFN-gamma and interleukin (IL)-12p40 in peripheral blood mononuclear cells isolated from healthy tuberculin reactors. Interestingly, IL-12p40 production in response to rMTSP11 was significantly higher than that in response to rMTSP17 or the three components of the antigen 85 complex. These results suggest that MTSP11 antigen should be further evaluated as a component of a subunit vaccine.—Authors’ Abstract


OBJECTIVE: To evaluate the protective efficacy of the fusion DNA vaccine (AM) encoding tubercle Ag85B and MPT64 in mice infected with Mycobacterium tuberculosis. METHODS: C57BL/6 mice were intramuscularly immunized with the DNA vaccines. The mice were challenged with 10(6) CFU H37Rv via lateral tail vein 35 days later after the third immunization for DNA vaccine groups and 100 days later for BCG vaccinated group. The mice in vaccinated groups and control groups were sacrificed 42 days later following challenge. The lungs and spleens were removed respectively, and the number of CFU in organs and histopathologic changes was determined. The antibody level, IFN-gamma, IL-4 and the survival time in all of the mice were evaluated. RESULTS: Antibody titer of pcDNA/Ag85B + pcDNA/MPT64 group and pcDNA/AM group was higher than that of other groups (p <0.05). The level of IFN-gamma produced by spleen lymphocytes and spleen lymphocyte proliferation from BCG group, pcDNA/Ag85B, pcDNA/Ag85B + pcDNA/MPT64 group and pcDNA/AM group was higher than that of other groups (p <0.05). No IL-4 was found in all groups. The number of bacterial colonies in the lungs and spleens was significantly decreased at 6th week postchallenge in all the vaccinated groups (p <0.05), especially in BCG group (p <0.01). The pulmonary histopathological changes were observed 6 weeks later following challenge with M. tuberculosis H37Rv. In PBS and pcDNA3.1 groups, the lesion was characterized by seroplastic inflammatory infiltration and lung tissue necrosis, in BCG group by granulomas and numerous macrophages, lymphocytes and a few epithelioid cells. The lesion in pcDNA/Ag85B groups was characterized by seroplastic inflammatory infiltration and a few macrophages, in pcDNA/Ag85B + pcDNA/MPT64 group and pcDNA/AM group, by granulomas, numerous macrophages and lymphocytes. The lesion in spleen was different from the lung and characterized by proliferative lymphocytes and inflammatory infiltration. The results in spleen were similar to those in lung. The survival time of BCG vaccinated mice after challenge with M. tuberculosis H37Rv was longer than that of other groups. The survival time of AM group was longer than that of other DNA vaccine
groups. CONCLUSION: The pcDNA/AM can improve the protective efficacy in immunized mice against M. tuberculosis.—Authors’ Abstract


Despite the availability of a vaccine for over 80 years, the tuberculosis epidemic continues to be a major cause of mortality and morbidity throughout the world. The factors contributing to the resurgence of tuberculosis and the possible explanations for the failure of the current vaccine, bacille Calmette-Guerin, are discussed. The nature of protective immunity to Mycobacterium tuberculosis and how this relates to the development of new candidate vaccines is then considered. The issues surrounding the progression of the most promising candidates into Phase I clinical trials are also discussed.—Authors’ Abstract


We report here the induction of specific protective cellular immunity against Mycobacterium tuberculosis by the employment of vaccination with recombinant attenuated Listeria monocytogenes strains. We constructed self-destructing attenuated L. monocytogenes Delta 2 strains carrying eukaryotic expression plasmids for the antigen 85 complex (Ag85A and Ag85B) and for MPB/MPT51 (mycobacterial protein secreted by M. bovis BCG/mycobacterial protein secreted by M. tuberculosis) molecules. Infection of these recombinant bacteria allowed expression of the genes in the J774A.1 murine macrophage cell line. Intraperitoneal vaccination of C57BL/6 mice with these recombinant bacteria was capable of inducing purified protein derivative-specific cellular immune responses, such as foot pad reactions, proliferative responses of splenocytes, and gamma interferon production from splenocytes, suggesting the efficacy of vaccination against mycobacterial infection by use of these recombinant L. monocytogenes strains. Furthermore, intravenous vaccination with recombinant bacteria carrying expression plasmids for Ag85A, Ag85B, or MPB/MPT51 in BALB/c mice elicited significant protective responses, comparable to those evoked by a live Mycobacterium bovis BCG vaccine. Notably, this is the first report to show that MPB/MPT51 is a major protective antigen in addition to Ag85A and Ag85B, which have been reported to be major mycobacterial protective antigens.—Authors’ Abstract


Alternate modalities for the treatment of Mycobacterium tuberculosis are needed due to the rise in numbers of immunosuppressed individuals at risk for serious disease and the increasing prevalence of multidrug-resistant isolates. Interleukin-12 (IL-12) has been shown to improve immune responses against M. tuberculosis infection in both humans and mice. Previous studies using high-dose IL-12 in various disease models reported a paradoxical immunosuppression. We demonstrate here that exogenous administration of IL-12 for 8 weeks after an aerosolized low dose of M. tuberculosis results in increased survival and decreased pulmonary bacterial loads for CD4-T-cell-deficient mice, most likely due to an early increase in gamma interferon. IL-12 treatment did not impair or enhance the ability of the wild-type mice to control infection, as measured by bacterial numbers. Two novel findings are reported here regarding exogenous IL-12 therapy for M. tuberculosis infections: (i). IL-12 treatment resulted in decreased numbers of immune cells and reduced frequencies of lymphocytes (CD8(+), CD4(+), and NK cells) in the lungs of infected mice and (ii). IL-12 therapy reduced the pathology of M.
tuberculosis-infected lungs, as granulomas were smaller and less numerous. These studies support an immunoregulatory role for IL-12 in tuberculosis.—Authors’ Abstract


Using plasmid vaccination with DNA encoding the putative phosphate transport receptor PstS-3 from Mycobacterium tuberculosis and 36 overlapping 20-mer peptides spanning the entire PstS-3 sequence, we determined the immunodominant Th1-type CD4(+) T cell epitopes in C57BL/10 mice, as measured by spleen cell IL-2 and IFN-gamma production. Furthermore, a potent IFN-gamma-inducing, D(b)-restricted CD8(+) epitope was identified using MHC class I mutant B6.C-H-2(bm13) mice and intracellular IFN-gamma and whole blood CD8(+) T cell tetramer staining. Using adoptive transfer of CFSE-labeled, peptide-pulsed syngeneic spleen cells from naive animals into DNA vaccinated or M. tuberculosis-infected recipients, we demonstrated a functional in vivo CTL activity against this D(b)-restricted PstS-3 epitope. IFN-gamma ELISPOT responses to this epitope were also detected in tuberculosis-infected mice. The CD4(+) and CD8(+) T cell epitopes defined for PstS-3 were completely specific and not recognized in mice vaccinated with either PstS-1 or PstS-2 DNA. The H-2 haplotype exerted a strong influence on immune reactivity to the PstS-3 Ag, and mice of the H-2(b, p, and f) haplotype produced significant Ab and Th1-type cytokine levels, whereas mice of H-2(d, k, r, s, and q) haplotype were completely unreactive. Low responsiveness against PstS-3 in MHC class II mutant B6.C-H-2(bm12) mice could be overcome by DNA vaccination. IFN-gamma-producing CD8(+) T cells could also be detected against the D(b)-restricted epitope in H-2(p) haplotype mice. These results highlight the potential of DNA vaccination for the induction and characterization of CD4(+) and particularly CD8(+) T cell responses against mycobacterial Ags. Authors’ Abstract


We developed a live, fully attenuated Mycobacterium tuberculosis vaccine candidate strain with two independent attenuating auxotrophic mutations in leucine and pantothenate biosynthesis. The deltaleuD delta-panCD double auxotroph is fully attenuated in the SCID mouse model and highly immunogenic and protective in the extremely sensitive guinea pig tuberculosis model, reducing both bacterial burden and disease pathology.—Authors’ Abstract


OBJECTIVE: To investigate the fused expression of secreted protein Ag85B-ESAT6 of Mycobacterium tuberculosis, and to provide a promising preventive subunit vaccine against tuberculosis. METHODS: The gene encoding Ag85B and ESAT6 protein was amplified by PCR from genome of Mycobacterium tuberculosis H(37)Rv strain, and inserted into cloning vector P(GEM)-T-easy, and into cloning vector P(PRO) EXHT, and the recombinant plasmid was transformed into expressible strain E. coli DH5 alpha, induced with IPTG and fusion protein was purified by Ni-NTA purification system. The specific antibody titer in the sera of BALB/c mouse immunized with two fusion protein was detected by ELISA. RESULTS: The sequences of Ag85B and ESAT6 by PCR amplification
were identical to those reported by GenBank. The recombinant plasmid fused expression protein of Ag85B-ESAT6 with relative molecular mass (Mr) of 37,000, which was confirmed by Western-blot analysis with specific monoclonal antibody against $6 \times$ HismAb. The fused expression protein was insoluble. It could be purified by Ni-NTA purification system. The specific antibody titer in the sera of BALB/c mouse immunized with fusion Ag85B-ESAT6 was 1:1000 and that of mouse immunized with fusion protein ESAT6-Ag85B was 1:5000. CONCLUSIONS: Secreted protein Ag85B-ESAT6 of Mycobacterium tuberculosis was successfully fused expressed in E. Coli DH5 alpha. It may become a new type of vaccine against tuberculosis.—Authors’ Abstract


DNA vaccine may be a promising tool for controlling tuberculosis development. However, vaccines encoding single antigens of mycobacterium did not produce protective effect as BCG did. In the present study, we evaluated the immunogenicity and protective efficacy of a divalent DNA vaccine encoding two immunodominant antigens Ag85B and MPT64 of Mycobacterium tuberculosis. We found that both humoral and Th1-type (high IFN-kappa, low IL-4) cellular responses obtained from the divalent DNA vaccine group were significantly higher than that conferred by BCG. RT-PCR results showed that antigens were expressed differentially in various organs in divalent DNA vaccine group. The survival rate for mice treated with the divalent DNA vaccine after challenging with high doses of virulent M. tuberculosis H37Rv was significantly higher than that of the BCG group or any of the single DNA vaccine group. Significant differences were also found between the single and divalent DNA vaccinated mice in terms of body, spleen and lung weight. Bacterial loading decreased about 2000-fold in lungs and about 100-fold in spleens of divalent DNA vaccinated mice when compared with that of the control group. We conclude that our divalent DNA vaccine may be a better choice for controlling tuberculosis disease in animals.—Authors’ Abstract

Epidemiology


OBJECTIVE: To study the profile of leprosy cases at Nkhotakota District Hospital in Central Region of Malawi. DESIGN: Retrospective cross-sectional study of all registered cases of leprosy from records over a nine year period (January 1992 to April 2001) SETTING: Nkhotakota District Hospital-Central Region of Malawi. RESULTS: In total 526 cases of leprosy were identified from the records. The prevalence rates gradually increased from 0.998 per 10,000 cases in 1992 to 3.39 cases per 10,000 in 1995. There was however a gradual decline of prevalence rates from 1997/1998 that had 3.17 cases per 10,000 to 1.3 cases per 10,000 in 2001. 1996 registered 2.34 cases per 10,000. Fifty seven cases (10.8%) were found with children of the age of 14 or below and 469 (89.2%) cases were of adults. Paucibacillary leprosy presented with more cases than multibacillary leprosy ($p < 0.0000001$). There were 80 (15.2%) cases of multibacillary leprosy compared to 446 (84.8%) cases of paucibacillary leprosy. In addition more males were affected by multibacillary leprosy than females ($p < 0.0001$) and females were more affected by paucibacillary leprosy ($p < 0.01$) than males. CONCLUSION: The results show that paucibacillary leprosy though minor in Malawi can become endemic as paucibacillary leprosy is a reflection of leprosy contacts in the population. We therefore
recommend continued epidemiological surveys of leprosy. Training in leprosy detection should be encouraged so that this disease can be totally eradicated in Malawi.—Authors’ Abstract


The incidence of clinical tuberculosis and clinical leprosy among household members of tuberculosis and leprosy patients in Sri Lanka was studied. The study period was approximately 20 years (January 1981 to December 2001) and the total number of patients and contacts were 325 and 968 for tuberculosis and 726 and 3066 for leprosy, respectively. While none of the tuberculosis patient households had more than 1 patient nor any contacts who developed clinical disease during the observation period, 20% (148/726) of the leprosy patients had more than 1 patient in the family and 0.9% (13/1403) of their contacts who were followed-up developed clinical leprosy during the observation period. Although the tuberculosis patient household contacts did not develop clinical disease, in 79% (88/112) of contacts who were tested by Western blot analysis, there was serologic evidence of *Mycobacterium tuberculosis* infection. These data show that in populations of comparable socio-economic, environmental and geographic locations, tuberculosis and leprosy show very different transmission patterns. In general, in tuberculosis household contacts, in spite of exposure, infection did not proceed to clinical disease. In contrast, a significant number of leprosy household contacts developed clinical leprosy. These findings have implications in the design and implementation of control programs for these two diseases.—Authors’ Abstract


A 19-year-old female patient of lepromatous leprosy with Type II reaction, on multidrug therapy and prednisolone, presented with acute onset flaccid quadriplegia. The cerebrospinal fluid examination revealed albumino-cytologic dissociation. Nerve biopsy showed infiltration with lepra bacilli, features of vasculitis, and demyelination. There were no other identifiable precipitating factors for Guillain Barre Syndrome in this patient. Her condition improved without any steroid therapy. This case emphasizes the hypothesis that cell injury caused by Type II reaction can expose neural antigens and incite an autoimmune reaction in the form of Guillain Barre Syndrome.—Authors’ Abstract


BACKGROUND: The concept of elimination of an infectious disease is different from eradication and in a way from control as well. In disease elimination programs the desired reduced level of prevalence is set up as the target to be achieved in a practical time frame. Elimination can be considered in the context of national or regional levels. Prevalence levels depend on occurrence of new cases and thus could remain fluctuating. There are no ready pragmatic methods to monitor the progress of leprosy elimination programs. We therefore tried to explore newer methods to answer these demands. With the lowering of prevalence of leprosy to the desired level of 1 case per 10,000 population at the global level, the program administrators’ concern will be shifted to smaller areas e.g., national and subnational levels. For monitoring this situation, we earlier observed that lot quality assurance sampling (LQAS), a quality control tool in industry was useful in the initially high endemic areas. However, critical factors such as geographical distribution of cases and adoption of cluster sampling design instead of simple random sampling design deserve attention before LQAS could
generally be recommended. The present exercise was aimed at validating applicability of LQAS, and adopting these modifications for monitoring leprosy elimination in Tamil Nadu state, which was highly endemic for leprosy. METHODS: A representative sample of 64,000 people drawn from eight districts of Tamil Nadu state, India, with maximum allowable number of 25 cases was considered, using LQAS methodology to test whether leprosy prevalence was at or below 7 per 10,000 population. Expected number of cases for each district was obtained assuming Poisson distribution. Goodness of fit for the observed and expected cases (closeness of the expected number of cases to those observed) was tested through chi(2). Enhancing factor (design effect) for sample size was obtained by computing the intraclass correlation. RESULTS: The survey actually covered a population of 62,157 individuals, of whom 56,469 (90.8%) were examined. Ninety-six cases were detected and this number far exceeded the critical value of 25. The number of cases for each district and the number of cases in the entire surveyed area both followed Poisson distribution. The intraclass correlation coefficients were close to zero and the design effect was observed to be close to one. CONCLUSIONS: Based on the LQAS exercises leprosy prevalence in the state of Tamil Nadu in India was above 7 per 10,000. LQAS method using clusters was validated for monitoring leprosy elimination in high endemic areas. Use of cluster sampling makes this method further useful as a rapid assessment procedure. This method needs to be tested for its applicability in moderate and low endemic areas, where the sample size may need increasing. It is further possible to consider LQAS as a monitoring tool for elimination programs with respect to other disease conditions.—Authors’ Abstract


Recent discovery of genetic diversity of *Mycobacterium leprae* such as variable number of tandem repeats opened a new era in molecular epidemiology of leprosy infection. It was revealed that the leprosy bacillus in residential environment of endemic villages is an important source of infection. The global elimination strategy will be revised taking new molecular epidemiological knowledge into account. Responsibility of leprosy specialist is to propose feasible control program to local administration based on the epidemiological analysis on transmission of the disease.—Authors’ Abstract


BACKGROUND: Leprosy is an important public health problem in many developing countries and many features of its determinants are still obscure. METHODS: To investigate whether the incidence of leprosy is related to certain environmental and socioeconomic determinants, an ecological study was undertaken in 165 municipalities of the state of Ceara, Brazil. Social, economic, education, sanitation, demography, meteorology, and health data were collected. The dependent variable was the average incidence rate of leprosy from 1991 to 1999. Simple and multiple linear regressions were performed to assess the relationship between the dependent and the independent variables. RESULTS: The average incidence rate for all the municipalities for the 1991–1999 period, varied from 0.06 to 14.68 per 10,000 persons per year. The level of inequality (beta = 1.67, p = 0.011), the mean years of study among the population ≥25 years old (beta = 1.35, p <0.001), the population growth from 1991 to 1996 (beta = 0.02, p = 0.007), the percentage of children 7–14 years old that did not go to the school (beta = 0.02, p = 0.028), and the presence of a railroad in the municipality (beta = 0.45, p = 0.038) were found to be predictors of the incidence rate of leprosy in Ceara. CONCLUSION: Our findings fit the assumption that, in Ceara, leprosy is associated with a high level of poverty and uncontrolled urbanization. We put forward the hypothesis that urbanization increases not
only social inequality eventually leading to strong polarization, but also excludes people from social and material opportunities. Apparently, such deprivations render them susceptible for leprosy.—Authors’ Abstract


Application of molecular biological techniques to the epidemiological study of leprosy is described. Studies of detecting Mycobacterium leprae DNA in samples of the nasal mucus are discussed in terms of the epidemiology and the significance of high prevalence. Epidemiological studies on the transmission of leprosy and correlation between geographic distribution of different M. leprae rpoT genotypes and prehistoric spread of the leprosy by genotyping based on the genomic polymorphism are introduced.—Authors’ Abstract


Trends in case detection and case detection rate (CDR) since 1985 are described at regional and national levels. Annual case detection by WHO Region was available for 1994–2000. Using different sources, complete time series for case detection were constructed for 1985–1998 for a group of 33 endemic countries cumulatively (top 33), and for 14 individual countries (top 14). Population statistics were used to derive CDRs. India contributed 79% to global case detection in 1998. Africa, the Americas and South-East Asia each contributed about 30% when India is excluded. During 1994–2000, case detection did not decrease in these three WHO Regions. The 33 countries contributed 99% and 98% to global case detection in 1994 and 1998, respectively. Cumulative case detection for the top 33 minus India gradually increased, overall almost doubling. The contribution of the top 14 to case detection of the top 33 hardly changed over time, equalling 96% in 1998 (81% when India is excluded). In terms of annual case detection, Brazil was always ranked second after India; it accounted for 27% of 1998 case detection in the top 33 except India. In 1998, seven of the top 14 countries—including India and Brazil—had CDRs above 2 per 10,000. The CDR did not exceed 1 per 10,000 for the other half. Decreasing tendencies in CDR, either for the whole period or in the 1990s, are observed for four of the top 14 countries (Guinea and three Western Pacific countries: China, Vietnam and the Philippines). In conclusion, there is no general decline in case detection to date, and several important countries still have high CDRs. Prevalence is an irrelevant indicator for monitoring epidemiological changes in leprosy. Trends in the transmission and incidence of leprosy are still completely unclear, necessitating further research. The target to eliminate leprosy as a public health problem, defined as a prevalence of less than 1 per 10,000, is therefore also an inadequate yardstick for decision making on leprosy control.—Authors’ Abstract


An innovative method that combined awareness creation with screening of high school students by their peers was undertaken in 26 randomly selected schools in the project area of the Schieffelin Leprosy Research and Training Center, Karigiri, Vellore, India. This method entailed educating teachers and student leaders in grades 8–12 about leprosy and how to suspect leprosy among their peers. The student leaders in turn conducted a similar awareness programme for their peers and encouraged them to report if they suffered from any skin problem or skin lesion. Based on the reporting by their peers, the class leaders prepared a ‘suspect list’. Within a fortnight of the awareness program, a trained leprosy worker visited the school and examined all the students on the ‘suspect list’. Those diagnosed to have leprosy were referred to a medical officer, who then confirmed the diagnosis and initiated treatment. Among the 23,125 students enrolled in the
26 randomly selected schools, 234 student leaders were educated about leprosy and trained to detect suspect lesions among their peers. A total of 2200 (9.5%) children reported with skin lesions to their leaders and after screening by a leprosy supervisor and confirmation by a medical officer, 14 new cases (NCDR 6.05/10,000) were detected. This rate was found to be comparable with case detection rates of annual school surveys done during the National Leprosy Eradication Programme (NLEP), when all schoolchildren were examined. The paper suggests that schoolchildren can be used effectively in leprosy case detection and this method has the additional advantage of creating awareness among them, their teachers and communities.—Authors’ Abstract

Rehabilitation


Stigmatization by the general population and their negative attitudes towards leprosy negatively impacts on patients’ mental health, and so too does patients’ perception of that stigma. The objective of this present study is to assess the depressive status of leprosy patients, the patient perception of that stigma, and its association with their depressive status in Dhaka, Bangladesh. Subjects were 140 patients, and a selected comparison group of 135 local people without any chronic diseases. To evaluate depressive status, the Center for Epidemiologic Studies Depression scale (CES-D) Bengali version was applied. The patient group’s depressive status was significantly more severe than that of the comparison group. Depressive status of those who answered affirmatively was significantly more severe than that of those who answered negatively for three responses to questions: 1) ‘I have been physically attacked by people’, 2) ‘I feel people regard me as strange’ and 3) ‘I have been refused the purchase of something by a shopkeeper’. The results showed that the depressive status in leprosy patients was greater than that of the general public. Further, actual experiences of discrimination based on stigma associated with the depressive status of leprosy patients. Mental health care for patients, regulation of discriminatory action and education that would decrease social stigma among the general population, especially people who might often have contact with patients, seem necessary to improve the mental health of Bangladeshi leprosy patients.—Authors’ Abstract

Other Mycobacterial Diseases


BACKGROUND: Mycobacterium chelonae and Mycobacterium fortuitum are the 2 most commonly implicated species of nontuberculous mycobacteria in cases of bacterial keratitis. OBJECTIVES: This article summarizes available data on the in vitro antibacterial activity against M. chelonae or M. fortuitum of 2 agents—amikacin and clarithromycin—that have been used in the treatment of bacterial keratitis. In addition, the article reviews the in vitro activity of 5 commercially available topical ocular fluoro-quinolones (in order of availability, ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin, and moxifloxacin) that may have potential in the surgical prophylaxis and treatment of keratitis caused by M. chelonae or M. fortuitum. METHODS: A search of the English-language literature indexed on the MEDLINE, Life Sciences, EMBASE, BIOSIS, and Pharmaprojects databases from 1966 to October 7, 2003, was conducted using the terms Mycobacterium chelonae, Mycobacterium fortuitum, bacterial keratitis, topical antibiotic therapy, ocular infection- mycobacteria, and LASIK infec-
tions. Data on the minimum concentrations at which 90% of isolates were inhibited (MIC(90)s) were reviewed and compared.

RESULTS: In the literature reviewed, the MIC(90) against *M. fortuitum* was from 1 to 16 microg/mL for amikacin, from ≤2 to ≥8 microg/mL for clarithromycin, from 0.1 to 1 microg/mL for ciprofloxacin, from 0.5 to 3.13 microg/mL for ofloxacin, and ≤2 microg/mL for levofloxacin. The results were similar against *M. chelonae*. The fourth-generation fluoroquinolones—gatifloxacin and moxifloxacin—had similar MIC(90)s against *M. fortuitum* (both, 0.2 to 1 microg/mL); however, moxifloxacin had greater activity than gatifloxacin against *M. chelonae* (minimum inhibitory concentration range: moxifloxacin, ≤1 to 1.6 microg/mL; gatifloxacin, 3.2 to 6.25 microg/mL). CONCLUSIONS: Topical fluoroquinolones may be beneficial for ocular surgical prophylaxis and for the treatment of keratitis caused by *M. chelonae* or *M. fortuitum*. Based on their reported MIC(90)s, none of the antibacterials reviewed had greater in vitro activity than moxifloxacin. In addition, moxifloxacin had greater in vitro activity than gatifloxacin against *M. chelonae*, one of the predominant nontuberculous mycobacterial species involved in bacterial keratitis. Pending the conduct of controlled clinical studies, these findings suggest that moxifloxacin may have utility in the prevention and treatment of atypical mycobacterial keratitis.—Authors’ Abstract


OBJECTIVE: Data about the characteristics of patients with the human immunodeficiency virus (HIV) and concomitant mycobacterial skeletal infection are scarce. Thus, our aim was to describe this condition in a cohort of 11 patients. METHODS: A review of the records of 11 HIV-positive individuals with microbiological confirmation of mycobacterial osteoarticular infection was conducted. The studied data included: age, sex, risk factor for the HIV days between the onset of symptoms and diagnosis, evidence of previous tuberculosis, location of the infection, isolated organism, diagnostic method, laboratory data (erythrocyte sedimentation rate, haemoglobin, leukocyte count), number of CD4+ lymphocytes, antiretroviral therapy, treatment and outcome. RESULTS: Eight patients were men and 3 were women. The median age was 34.2 years (range 20 to 46 years). Previous tuberculosis was present in 5 cases. Mean days between the onset of symptoms and diagnosis was 124 (range 20 to 365 days). Infections involved the knee (4 cases), spine

INTRODUCTION: *Mycobacterium fortuitum* skin infections are rare and usually iatrogenic. We report a case with cervical involvement following a facelift. OBSERVATION: A 65 year-old woman, without past history, underwent bilateral surgical facelift, complicated by cutaneous necrosis and treated with directed healing at home. Six weeks later, an abscessed nodule appeared under the left maxillary and was drained surgically. Then other pre-auricular and left cervical inflammatory nodules appeared without adenopathy or fever. *M. fortuitum* was isolated in bacteriological samples. The initially probabilistic antibiotherapy with carithromycin, subsequently adapted with amikacin and ciprofloxacin and then imipeneme for a total duration of 3 months, led to the clinical cure. DISCUSSION: *Mycobacterium fortuitum* is a rapidly growing, ubiquitous, mycobacteria responsible for nosocomial infections in immunocompetent patients, notably following plastic surgery. Contamination occurs where there has been a rupture in the skin barrier through contact with a vector (water, surgical material, antiseptic). Treatment, which is not codified, consists in the association of surgery and antibiotics for several months.—Authors’ Abstract

(3 cases), hip (2 cases), elbow (1 case) and tibia (1 case). ESR was frequently elevated. The CD4 count ranged from 0.03 to 0.779 × 10(9)/l (mean 0.245 × 10(9)/l). *M. tuberculosis* was the responsible organism in 9 cases, *Mycobacterium tuberculosis* plus *Staphylococcus aureus* in one case and *M. Kansasi* in one case. Patients received specific treatments with good results. Surgery was necessary in 4 cases. No deaths occurred. Four patients were anti-retroviral naive at the moment the diagnosis was made. The remainder 8 were on zidovudine therapy. CONCLUSION: The immunologic status of patients with HIV and concomitant mycobacterial skeletal infections is quite variable. The outcome of this condition seems to be good.—Authors’ Abstract


Sporotrichoid forms of atypical mycobacterial infections usually do not show a tendency to spontaneous healing. The therapy of choice in such cases is systemic antibiotics. We report three cases of sporotrichoid atypical mycobacterial infections of the skin which healed completely under long-term monotherapy with modern antibiotics (levofloxacin, clarithromycin, minocycline). We recommend confirming the diagnosis by means of culture, followed by monotherapy with low side-effect antibiotics, based on sensitivity studies. If pathogen proof is obtained by PCR, antibiotic therapy should be based on the known sensitivity of the pathogen in question.—Authors’ Abstract


A real-time PCR assay was developed to diagnose and identify the causative agents of suspected mycobacterial lymphadenitis. Primers and probes for the real-time PCR were designed on the basis of the internal transcribed spacer sequence, enabling the recognition of the genus *Mycobacterium* and the species *Mycobacterium avium* and *M. tuberculosis*. The detection limit for the assay was established at 1100 CFU/ml of pus, and the specificity tests showed no false-positive reaction with other mycobacterial species and other pathogens causing lymphadenitis. From 67 children with suspected mycobacterial lymphadenitis based on a positive mycobacterial skin test, 102 samples (58 fine-needle aspirates [FNA] and 44 tissue specimens) were obtained. The real-time PCR assay detected a mycobacterial infection in 48 patients (71.6%), whereas auramine staining and culturing were positive for 31 (46.3%) and 28 (41.8%) of the patients. The addition of the real-time PCR assay to conventional diagnostic tests resulted in the recognition of 13 more patients with mycobacterial disease. These results indicate that the real-time PCR is more sensitive than conventional staining and culturing techniques (p = 0.006). The *M. avium*-specific real-time PCR was positive for 38 patients, and the *M. tuberculosis*-specific real-time PCR was positive for 1 patient. Analysis of 27 patients from whom FNA and tissue biopsy specimens were collected revealed significantly more positive real-time PCR results for FNA than for tissue biopsy specimens (p = 0.003). Samples from an age-matched control group of 50 patients with PCR-proven cat scratch disease were all found to be negative by the real-time PCR. We conclude that this real-time PCR assay with a sensitivity of 72% for patients with lymphadenitis and a specificity of 100% for the detection of atypical mycobacteria can provide excellent support for clinical decision making in children with lymphadenitis.—Authors’ Abstract

AIM: To describe the radiological appearances of immune reconstitution inflammatory syndrome (IRIS) in human immunodeficiency virus (HIV)-infected patients with mycobacterial infections starting highly active anti-retroviral therapy (HAART). MATERIALS AND METHODS: Five consecutive HIV infected patients with IRIS due to mycobacterial infection were studied. Intercurrent infection and poor drug compliance were excluded as causes of presentation. The chest radiological appearances at the time of starting HAART and at the time of diagnosis of IRIS were compared. RESULTS: In these five patients there was clinical and radiological deterioration, occurring between 10 days and 7 months after starting HAART, leading to unmasking of previously undiagnosed mycobacterial infection or to worsening of mycobacterial disease. All five patients had HAART-induced increases in CD4+ T lymphocyte counts and reductions in peripheral blood HIV “viral load.” Chest radiographic abnormalities due to IRIS included marked mediastinal lymphadenopathy in three patients—severe enough to produce tracheal compression in two patients (one of whom had stridor)—and was associated with new pulmonary infiltrates in two patients. The other two patients had new infiltrates, which in one patient was associated with a pleural effusion. CONCLUSION: These cases illustrate the diverse chest radiographic appearances of IRIS occurring after HAART in patients with mycobacterial and HIV co-infection. Marked mediastinal lymphadenopathy occurred in three of these five patients (with associated tracheal narrowing in two patients); four patients developed pulmonary infiltrates and one had an effusion. The cases further highlight that the onset of IRIS may be delayed for several months after HAART is started.—Authors’ Abstract


*Mycobacterium celatum* is a recently described organism. Herein we describe a case of *M. celatum* lymphadenitis in an immunocompetent child and the first reported case of a *M. celatum* infection (lung abscess) in a transplant recipient. A literature review identified 19 other cases of *M. celatum* infection. Fifteen occurred in patients with the acquired immunodeficiency syndrome. Of these, nine were disseminated and six were localized (primarily to the lungs). The remaining four patients were immunocompetent and had localized infection (pneumonitis or lymphadenitis). Diagnosis of *M. celatum* infection can be challenging as *M. celatum* can cause false-positive results with the current version of the Amplified *Mycobacterium tuberculosis* Direct Test (Gen-Probe, San Diego, CA.). Definitive identification is available by DNA sequencing or high-performance liquid chromatography. *M. celatum* can cause infection in immunocompromised and immunocompetent hosts.—Authors’ Abstract


The Infectious Diseases Working Party of the European Blood and Marrow Transplant Group conducted a survey to obtain information about the frequency, presentation, and treatment of mycobacterial infection (MBI) in stem cell transplant (SCT) recipients. Among 29 centers, MBI was diagnosed in 0.79% of 1513 allogeneic and 0.23% of 3012 autologous SCT recipients during 1994–1998 a median of 160 days after transplantation. The mean interval between first symptoms and diagnosis was 29 days and was still longer for patients with atypical MBI or recipients of corticosteroid therapy. The prevalence of MBI was highest among those who received matched unrelated or mismatched SCTs from related donors. Of 31 patients, 20 had tuberculosis, 8 had atyp-
ical MBI, and 3 had diagnoses based on histological findings only. Five patients (16%) died, all of whom had received an allogeneic SCT. Because of the increased numbers of unmatched donors and transplantation programs in countries with a high prevalence of tuberculosis, constant vigilance is required to early detect MBI in SCT recipients.—Authors’ Abstract


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


Mycobacterial cervical lymphadenopathy is relatively uncommon in the United King-
transplant recipients. Issues involved in the management of NTM disease in transplant recipients are characterized by a case of disseminated infection due to *Mycobacterium avium* complex in a lung transplant recipient, with a review of the relevant literature.—Authors’ Abstract

**Duttaroy, B., Agrawal, C., and Pendse, A.**


There has been an increase in disease caused by Non Tuberculous Mycobacteria (NTM) since the early 1980s. Though ubiquitous in environment, they may act as clinically important pathogens in various conditions. More importantly they are resistant to the conventional anti-tuberculous therapy (ATT) and respond to antibiotics such as quinolones and aminoglycosides and need an aggressive surgical intervention. Missing these atypical mycobacteria may lead to unnecessary administration of ATT and hence delay in proper management of the case. We report a case of spinal tuberculosis due to a Non Tuberculous Mycobacteria, *M. fortuitum* (Rapid grower). Relevant literature is also reviewed.—Authors’ Abstract

**Edmonson, B. C., Morris, W. R., and Osborn, F. D.**


We report the clinical and histopathologic findings of bacillary angiomatosis involving the palpebral conjunctiva with concomitant infection by cytomegalovirus and Mycobacterium species in a patient with acquired immune deficiency syndrome. After debulking, the conjunctival tissue was studied with the use of light and electron microscopy; stains for bacteria, acid-fast bacilli, and Bartonella species; and immunohistochemical studies for cytomegalovirus and herpes simplex virus. We observed the typical histopathologic findings of bacillary angiomatosis, the presence of bacilli stained by the Steiner and Steiner method, and the electron microscopic demonstration of bacilli consistent with Bartonella species. Immunohistochemistry confirmed infection with cytomegalovirus, which had been suggested by characteristic cytologic abnormalities. Acid-fast bacilli were also found in the excised tissue. Patients with bacillary angiomatosis of the conjunctiva may have infections with multiple additional microorganisms.—Authors’ Abstract

**Ergan, B., Coplu, L., Alp, A., and Artvinli, M.**


*Mycobacterium smegmatis* is a non-tuberculous mycobacterium that is usually associated with soft tissue or wound infections in humans. Pulmonary infections secondary to this pathogen are rarely seen and occur only in patients with an underlying condition, such as lipid pneumonia. This report presents the first case of *M. smegmatis* pneumonia in an otherwise healthy individual who had no predisposing condition.—Authors’ Abstract


We report two cases of lower-extremity furunculosis caused by *Mycobacterium mageritense*. Both patients were patrons of the same nail salon, where they received footbaths prior to pedicures. *M. mageritense* bacteria isolated from two whirlpool footbaths were determined to be closely related to the patient isolates by pulsed-field gel electrophoresis.—Authors’ Abstract

**Henry, M. T., Inamdar, L., O’Riordain, D., Schweiger, M., and Watson, J. P.**

Recent international guidelines published in 1997 and 1999 have proposed diagnostic and treatment criteria for disease caused by nontuberculous mycobacteria (NTM). In this paper, the epidemiological data, diagnostic criteria, treatment regimens and outcomes from 117 HIV-negative patients who had a positive culture for NTM between 1995–1999 are reviewed. The authors wished to identify factors associated with improved outcome in these patients. A total of 71 patients were believed to have a clinical disease caused by NTM, as defined by international criteria. A total of 72% patients were found to have had pulmonary disease. There was a rise in infections between 1995–1999, with a peak in infections in 1997. The most striking rise was in Mycobacterium avium intracellulare complex infections (1995: 33% infections; 1996: 36% infections; 1997: 41% infections; 1998: 61% infections; 1999: 57% infections). There was a link between deprivation and number of positive NTM isolates (34.4% isolates occurred in the areas of lowest Carstairs deprivation index versus 20.6% isolates from areas of least deprivation). There was a significant association between appropriate therapy, defined by American Thoracic Society and British Thoracic Society guidelines, and successful outcome (74%) in contrast to those who received inappropriate treatment prior to the publication of these guidelines. Nontuberculous mycobacteria infections remain a significant problem in non-HIV patients. Adherence to published guidelines may improve patient outcomes.—Authors’ Abstract


This is the first report of infection caused by “Mycobacterium lactica,” a rapidly growing, scotochromogenic mycobacterium that was isolated from the blood of an immunosuppressed child. The organism was identified by sequence analysis of >1400 bp of the 16S rRNA gene. The clinical relevance of this isolate, coupled with its unique 16S rRNA gene sequence, should prompt further investigation to establish this organism as a valid mycobacterial species.—Authors’ Abstract


Mycobacterium chelonae is a rapidly growing atypical mycobacterium that is a normal commensal of water and soil. We report a case of a 61-year-old man with seronegative rheumatoid arthritis and fibrosing alveolitis on long-term prednisolone
who presented with a number of tender, red, subcutaneous nodules on his upper arms and a pustule on his left cheek. Histopathologic examination revealed dense neutrophilic collections within the deep dermis and subcutaneous fat with abscess formation. Long filamentous organisms were seen within these collections and were subsequently identified by special stains and PCR as *Mycobacterium chelonae*. Treatment was not possible as the patient developed bacteria bronchopneumonia before identification of the organism and he subsequently died. Post-mortem revealed no extra-cutaneous evidence of mycobacterium infection.—Authors’ Abstract


Before highly active antiretroviral therapies (HAART) were available for the treatment of persons with HIV infection, disseminated *Mycobacterium avium*-intracellulare complex (MAC) infection was one of the most common opportunistic infections that affected people living with AIDS. Routine use of chemoprophylaxis with a macrolide has been advocated in guidelines for the treatment of HIV-infected individuals if they have a circulating CD4+ cell count of ≤50 cells/microL. In addition, lifelong prophylaxis for disease recurrence has been recommended for those with a history of disseminated MAC infection. The introduction of HAART has resulted in a remarkable decline in the incidence of opportunistic infections and death among persons living with AIDS. Considerable reconstitution of functional immune responses against opportunistic infections can be achieved with HAART. In the case of infection with MAC, there has been a substantial reduction in the incidence of disseminated infections in the HAART era, even in countries where the use of MAC prophylaxis was never widely accepted. Moreover, the clinical picture of MAC infections in patients treated with potent antiretroviral therapies has shifted from a disseminated disease with bacteraemia to a localised infection, presenting most often with lymphadenopathy and osteomyelitis. Data from several recently conducted randomised, double-blind, placebo-controlled trials led to the current practice of discontinuing primary and secondary prophylaxis against disseminated MAC infections at stable CD4+ cell counts >100 cells/microL. These recommendations are still conservative as primary or secondary disseminated MAC infections are only rarely seen in patients who respond to HAART, despite treatment initiation at very low CD4+ cell counts. Potential adverse effects of macrolide therapy and drug interactions with antiretrovirals also metabolised via the cytochrome P450 enzyme system must be critically weighed against the marginal benefit that MAC prophylaxis may provide in addition to treatment with HAART. These authors feel that, unless patients who initiate HAART at low CD4+ cell counts do not respond to HIV-treatment, routine MAC prophylaxis should not be recommended. Nevertheless, the patient population for whom MAC prophylaxis may still be indicated in the era of HAART needs to be identified in prospectively designed clinical trials.—Authors’ Abstract


The aim of this paper is to describe a rare case of vertebral osteomyelitis caused by *Mycobacterium flavescens* in an immunocompetent patient. *Mycobacterium flavescens* is a rapidly growing, pigmented, non-tuberculous mycobacterium, usually described as non-pathogenic for humans but occasionally reported as the cause of serious infectious complications recognized in clinical practice. This study stressed the importance of recent reports that describe the occurrence of vertebral osteomyelitis due to non-tuberculous mycobacteria that have also been recognized with an increasing incidence among immunocompetent hosts. A brief review of the current literature on human disease caused by *Mycobacterium flavescens* is also reported.—Author’s Abstract

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


Mycobacterium xenopi is very rare pathogen in Japan. We reported herein four cases of M. xenopi pulmonary disease. These patients were all male and their ages ranged from 53 to 72. They all had past history of pulmonary tuberculosis, including two cases who had been also treated for Mycobacterium kansasii pulmonary disease later. The bacilli could be cultured in Mycobacteria Growth Indicator Tube (MGIT) system from 10 sputum samples, but they could not be cultured on Ogawa egg media except for two samples. All four cases fulfilled the criteria for the diagnosis of nontuberculous mycobacteria pulmonary disease proposed by the Japanese Society for Tuberculosis. Combination chemotherapy including isoniazid, rifampicin, and ethambutol was started in all four cases when mycobacteria were detected under tentative diagnosis of the relapse of tuberculosis or Mycobacterium kansasii disease. Sputum converted to culture negative by the chemotherapy in two cases. In one case, the chemotherapeutic regimen was changed to rifampicin, ethambutol, and clarithromycin after the bacteriological identification of M. xenopi, and the new regimen was found to be effective. In the final case, both of the regimens were finally ineffective.—Authors’ Abstract

In March 2003, the New Jersey Department of Health and Senior Services (NJDHSS) was notified about three patients who acquired surgical-site infections caused by Mycobacterium chelonae after having face lifts (i.e., rhytidectomies) performed at an outpatient surgical center. NJDHSS learned subsequently of another patient with M. chelonae infection who had a rhytidectomy performed at a second surgical center. The four patients received diagnoses of M. chelonae infection during March 2002–February 2003. NJDHSS conducted an epidemiologic, environmental, and microbiologic investigation. This report summarizes the results of that investigation, which identified contaminated methylene blue used as a tissue-marking agent as the source of infection. Surgeons should use only sterile, single-use, tissue-marking agents during procedures that require aseptic technique, and clinicians should consider M. chelonae when evaluating surgical-site infections.—Authors’ Abstract


Buruli ulcer disease (BUD) is an emerging disease caused by Mycobacterium ulcerans. In the present study we have characterized the serological reactivities of sera from volunteer case patients with laboratory-confirmed BUD and controls living in three different regions of Ghana where the disease is endemic to determine if serology may be useful for disease confirmation. Our results showed highly reactive immunoglobulin G (IgG) responses among patients with laboratory-confirmed disease, healthy control family members of the case patients, and sera from patients with tuberculosis from areas where BUD is not endemic. These responses were represented by reactivities to multiple protein bands found in the M. ulcerans culture filtrate (CF). In contrast, patient IgM antibody responses to the M. ulcerans CF (MUCF) proteins were more distinct than those of healthy family members living in the same village. A total of 84.8% (56 of 66) of the BUD patients exhibited strong IgM antibody responses against MUCF proteins (30, 43 and 70 to 80 kDa), whereas only 4.5% (3 of 66) of the family controls exhibited such responses. The sensitivity of the total IgM response for the patients was 84.8% (95% confidence interval [CI], 74.3 to 91.6%), and the specificity determined with sera from family controls was 95.5% (95% CI, 87.5 to 98.4%). These studies suggest that the IgM responses of patients with BUD will be helpful in the identification and production of the M. ulcerans recombinant antigens required for the development of a sensitive and specific serological assay for the confirmation of active BUD.—Authors’ Abstract


We present a case of Mycobacterium avium-intracellulare (MAI) infection of the ankle joint in a patient with HIV infection. The patient presented with a painful, destructive arthropathy of the ankle. Initial microbiological studies were negative but infection with MAI was later identified from biopsies taken during hindfoot fusion. Antibiotic triple therapy was given and the patient remains pain-free without evidence of active infection. To our knowledge, this is the first case of MAI infection of the ankle reported in the literature. A high index of suspicion of (atypical) Mycobacterial infection should be maintained in patients with HIV infection presenting with an indolent but destructive arthropathy of the ankle joint.—Authors’ Abstract


This report presents two cases of cervical lymphadenitis due to Mycobacterium interjectum in healthy young children, identified by sequencing of the 16S rRNA gene. Surgical resection combined with chemotherapy resulted in cure. CONCLUSION: The attention of clinicians needs to be drawn to an emerging mycobacterial pathogen which might be overlooked or misidentified in routine laboratory testing.—Authors’ Abstract


We describe the first documented spillover of bovine tuberculosis from animals into the human population of the United Kingdom since the resurgence of the disease in cattle in the country. This finding suggests that there may be a small risk for transmission to humans, making continued vigilance particularly necessary.—Authors’ Abstract

We report on a patient with an abdominal wall abscess that developed after an inguinal hernia repair that utilized synthetic mesh. *Mycobacterium goodii*, a recently recognized, rapidly growing mycobacterium related to *M. smegmatis*, was isolated both from the abdominal wall aspirate and from surgically drained material. Infection resolved following thorough debridement, mesh removal, and prolonged antimicrobial therapy. This case report extends our understanding of the spectrum of *M. goodii* infection.—Authors’ Abstract


A 68-year-old man with adult-onset diabetes mellitus suffered an accidental puncture wound to the palm of his hand while playing with his pet dog. He received cephalosporin prophylaxis for 1 week. No inflammation occurred. Six months later, a mass developed near his elbow. It was removed. Histopathology revealed granulomas containing acid-fast bacilli (AFB). No culture was done. Swelling and decreased motion of the wrist and fingers developed. Magnetic resonance imaging revealed inflammation of the flexor compartment of the hand, wrist, and forearm. Surgical incision and drainage yielded purulent material, granulomatous inflammation, with AFB. Cultures yielded *Mycobacterium kansasii*. Several surgical procedures were required; *M. kansasii* was recovered. He received isoniazid and rifampin for 1 year and prolonged rehabilitation. After 4 years, he was relatively asymptomatic, with good function of wrist and fingers. We believe this to be the first report of tenosynovitis caused by *M. kansasii* in association with a dog bite.—Authors’ Abstract


A 41-year-old woman was admitted to our hospital because of fever and polyarthralgia. A diagnosis of systemic lupus erythematosus (SLE) was made based on the findings of polyarthritis, leukocytopenia, lymphocytopenia, proteinuria, and positive reactions for antinuclear antibody (ANA) and anti-double strand (ds)DNA antibody. She had also been suffering from a pulmonary *Mycobacterium avium* complex (MAC) infection with such symptoms as cough and sputum for the past 3 years. Antimicrobial drugs for MAC infection were administered first, and later she was given cyclophosphamide pulse therapy, consisting of methylprednisolone (8 mg/day) and mizoribine (100 mg/day). Owing to these therapeutic regimens, SLE was successfully treated without an exacerbation of the MAC infection. The risk factors for MAC infection and SLE are also discussed.—Authors’ Abstract


A pigmented, slowly growing *Mycobacterium avium* complex AccuProbe-positive organism was isolated from the sputum and pleural fluid of a 72-year-old female with bronchiectasis. The unusual morphology of the organism prompted further identification by 16S rRNA gene sequencing, revealing a perfect identity with previously uncharacterized strain Mycobacterium sp. MCRO 8 (GenBank accession no. X93034), with the closest established species by 16S rDNA analysis being *Mycobacterium interjectum*. HPLC of the organism corresponded to previously obtained patterns identified as *M. interjectum*-like and, upon sequence evalu-
tion of a selection of strains with a similar profile, more were subsequently identified as MCRO 8. A total of 16 strains isolated from human respiratory samples were evaluated in the characterization of this novel species, for which the name *Mycobacterium saskatchewanense* sp. nov. is proposed. The type strain is strain 00-250(T) (=ATCC BAA-544(T)=DSM 44616(T)=CIP 108114(T)).—Authors’ Abstract


A case of recurrent *Mycobacterium xenopi* infection presenting as Pott’s disease in a patient receiving etanercept for severe rheumatoid arthritis is described. A 49-yr-old Caucasian male had received a total of 11 months of anti-mycobacterial therapy for hip infection acquired 15 months earlier; he presented with progressive back pain, which was diagnosed as Pott’s disease. He had been treated with etanercept in addition to his prior immunosuppressive agents after the diagnosis of hip infection.—Authors’ Abstract

**Molecular and Genetic Studies**


The bacterial NusA protein enhances transcriptional pausing and termination and is known to play an essential role in antitermination. Antitermination is signaled by a nut-like cis-acting RNA sequence comprising boxB, boxA, and boxC. In the present study, we demonstrate a direct, specific high-affinity interaction between the rrn leader nut-like sites and the NusA proteins of *Mycobacterium tuberculosis* and *Escherichia coli*. This NusA-RNA interaction relies on the conserved region downstream of boxA, the boxC region, thus demonstrating a key function of this element. We have established an in vivo assay for antitermination in mycobacteria and use this to show that the *M. tuberculosis* rrn nut-like site enhances transcriptional read-through of untranslated RNA consistent with an antitermination signal within this site. Finally, we present evidence that this NusA-RNA interaction affects transcriptional events further downstream.—Authors’ Abstract


The pyrazinamidase gene coding for the enzyme that activates the bactericidal drug pyrazinamide contains a polymorphic site that is preserved in *Mycobacterium bovis*. We synthesized two sets of primers, one encompassing a 180 bp fragment, and the second spanning a 726 bp fragment including the full pncA gene. Following PCR of *Mycobacterium tuberculosis* and *M. bovis* samples, it is possible to discriminate by this polymorphism between these species by digestion with Eco065 I. Digestion of the 180 bp fragment results in two fragments of 101 and 79 bp, specific for *M. tuberculosis*. Alternatively, digestion of the 726 bp fragment yields three fragments of 452, 165 and 109 bp for *M. tuberculosis*, but only two fragments of 561 and 165 bp for *M. bovis*.—Authors’ Abstract

Repetitive-sequence-based PCR (rep-PCR) is useful for generating DNA fingerprints of diverse bacterial and fungal species. Rep-PCR amplicon fingerprints represent genomic segments lying between repetitive sequences. A commercial system that electrophoretically separates rep-PCR amplicons on microfluidic chips, and provides computer-generated readouts of results has been adapted for use with Mycobacterium species. The ability of this system to type \textit{M. tuberculosis} and \textit{M. avium} complex (MAC) isolates was evaluated. \textit{M. tuberculosis} strains (N = 56) were typed by spoligotyping with rep-PCR as a high-resolution adjunct. Results were compared with those generated by a standard approach of spoligotyping with IS6110-targeted restriction fragment length polymorphism (IS6110-RFLP) as the high-resolution adjunct. The sample included 11 epidemiologically and genotypically linked outbreak isolates and a population-based sample of 45 isolates from recent immigrants to Seattle, Wash., from the African Horn countries of Somalia, Eritrea, and Ethiopia. Twenty isolates exhibited unique spoligotypes and were not analyzed further. Of the 36 outbreak and African Horn isolates with nonunique spoligotypes, 23 fell into four clusters identified by IS6110-RFLP and rep-PCR, with 97% concordance observed between the two methods. Both approaches revealed extensive strain heterogeneity within the African Horn sample, consistent with a predominant pattern of reactivation of latent infections in this immigrant population. Rep-PCR exhibited 89% concordance with IS1245-RFLP typing of 28 \textit{M. avium} subspecies avium strains. For \textit{M. tuberculosis} as well as \textit{M. avium} subspecies avium, the discriminative power of rep-PCR equaled or exceeded that of RFLP. Rep-PCR also generated DNA fingerprints from \textit{M. intracellulare} (N = 8) and MAC(x) (N = 2) strains. It shows promise as a fast, unified method for high-throughput genotypic fingerprinting of multiple Mycobacterium species.—Authors’ Abstract


OBJECTIVE: To prepare the recombinant CFP10-ESAT-6 fusion protein, and to study its immunological characteristics, and its potential for serodiagnosis of tuberculosis. METHODS: The lhp-ESAT-6 fusion gene was amplified by Gene SOEing, and then cloned into pQE30 plasmid. The recombinant CFP10-ESAT-6 fusion protein was expressed and purified. Its antigenicity was confirmed by Western blot. Animal models infected with \textit{M. tuberculosis} H(37)Rv strain and \textit{M. bovis} BCG respectively were made to evaluate the potential value of the fusion protein in the serodiagnosis of tuberculosis. RESULTS: The sequence of recombinant plasmid pQE30-CFP10-ESAT-6 was identical to the predicted sequence. The recombinant protein (rCFP10-ESAT-6), about 26,000, existed in the cytoplasm of DH5alpha in soluble form and represented 40% of the total bacterial protein. The purity and concentration of the final product was 98% and 1.2 g/L, respectively. Western blot showed that the rCFP10-ESAT-6 had good immunoreactivity with sera from patients with active tuberculosis and rabbits immunized with CFP10 and ESAT-6 respectively. The positive cutoff value was A(490) plus 2 standard deviation from negative guinea pig sera detected by ELISA. Serological reactivity to rCFP10-ESAT-6 was observed in 11 of the serum samples from guinea pigs with tuberculosis and 1 of sera from guinea pigs infected with BCG, while the serological reactivity to PPD was observed in 11 of sera from guinea pigs with tuberculosis and in 11 of sera from guinea pigs infected with BCG. CONCLUSIONS: The rCFP10-ESAT-6 fusion protein was highly expressed in soluble form in E. coli. It had antigenicity of both CFP10 and ESAT-6, and could be used to differentiate infection with \textit{M. tuberculosis} H(37)Rv strain from immunization with \textit{M. bovis} BCG. The study provided experimental data for potential application of rCFP10-ESAT-6 in the diagnosis of tuberculosis.—Authors’ Abstract

Delogu, G., Pusceddu, C., Bua, A., Fadda, G., Brennan, M. J., and Zanetti, S.

Identification of the novel PE multigene family was an unexpected finding of the genomic sequencing of *Mycobacterium tuberculosis*. Presently, the biological role of the PE and PE_PGRS proteins encoded by this unique family of mycobacterial genes remains unknown. In this report, a representative PE_PGRS gene (Rv1818c/PE_PGRS33) was selected to investigate the role of these proteins. Cell fractionation studies and fluorescence analysis of recombinant strains of *Mycobacterium smegmatis* and *M. tuberculosis* expressing green fluorescent protein (GFP)-tagged proteins indicated that the Rv1818c gene product localized in the mycobacterial cell wall, mostly at the bacterial cell poles, where it is exposed to the extracellular milieu. Further analysis of this PE_PGRS protein showed that the PE domain is necessary for subcellular localization. In addition, the PGRS domain, but not PE, affects bacterial shape and colony morphology when Rv1818c is overexpressed in *M. smegmatis* and *M. tuberculosis* expressing green fluorescent protein (GFP)-tagged proteins indicated that the Rv1818c gene product localized in the mycobacterial cell wall, mostly at the bacterial cell poles, where it is exposed to the extracellular milieu. Further analysis of this PE_PGRS protein showed that the PE domain is necessary for subcellular localization. In addition, the PGRS domain, but not PE, affects bacterial shape and colony morphology when Rv1818c is overexpressed in *M. smegmatis* and *M. tuberculosis*. Taken together, the results indicate that PE_PGRS and PE proteins can be associated with the mycobacterial cell wall and influence cellular structure as well as the formation of mycobacterial colonies. Regulated expression of PE genes could have implications for the survival and pathogenesis of mycobacteria within the human host and in other environmental niches.—Authors’ Abstract


Analysis of heat shock protein 65 (hsp65) gene restriction fragment length polymorphism (RFLP) is done frequently to identify non-tuberculous mycobacteria (NTM) on a genetic basis. Here we report the results of analysing the hsp65 patterns of some rarely isolated NTM for which patterns have not been published before (*Mycobacterium bohemicum*, *Mycobacterium hassiacum*, *Mycobacterium heckeshornense*, *Mycobacterium monacense*, and *Mycobacterium tripplex*). Furthermore new hsp65-variants
for Mycobacterium interjectum (type II), Mycobacterium mucogenicum (type V), Mycobacterium gordoneae (type VIII) and Mycobacterium paraffinicum (perhaps synonymous to Mycobacterium scrofulaceum) are described. All species were characterised by hsp65-RFLP, sequencing a 441-bp fragment of the hsp65 gene and sequencing the hypervariable region of the 16S rDNA. Additional data for less frequently isolated mycobacteria are provided and the hitherto described data for the Mycobacterium gordonae complex are summarised. Although the hsp65-RFLP analysis turned out to be a useful method a number of restraints (lack of standardisation, slight variability in fragment length) limits its broader use. Reliable identification of NTM needs, however, more than one molecular method. Identification results obtained by applying different methods yielded conflicting results. Confusion may be caused by older data base entries which are not updated and not longer reflect the actual systematic and taxonomy of the genus Mycobacterium.—Authors’ Abstract


We describe a novel, simple, rapid, and highly sensitive method to detect single-nucleotide polymorphisms (SNPs) in Mycobacterium tuberculosis and other organisms. Amplification refractory mutation (ARMS) SNP assays were modified by converting the SNP-detecting linear primers in the ARMS assay to hairpin-shaped primers (HPs) through the addition of a 5′ tail complementary to the 3′ end of the linear primer. The improved ability of these primers to detect SNPs in M. tuberculosis was compared in a real-time PCR with SYBR-I green dye. Linear primers resulted in incorrect or indeterminate allele designation for 6 of the 13 SNP alleles tested in seven different SNP assays, while HPs determined the correct SNP in all cases. We compared the cycle threshold differences (DeltaC(t)) between the reactions containing primer-template matches and the reactions containing primer-template mismatches (where a larger DeltaC(t) indicates a more robust assay). The use of HPs dramatically improved the mean DeltaC(t) values for the SNP assays (7.6 for linear primers and 11.2 for HPs). We designed 98 different HP assays for SNPs previously associated with resistance to the antibiotic isoniazid to test the large-scale utility of the HP approach. Assay design was successful in 72.4%, 83.7%, 88.8%, and 92.9% of the assays after one to four rounds of assay design, respectively. HP SNP assays are simple, sensitive, robust, and inexpensive. These advantages favor the application of this technique for SNP assays of M. tuberculosis and other organisms.—Authors’ Abstract


Although Mycobacterium marinum and Mycobacterium tuberculosis are very closely related they differ significantly in their growth rates. The Type strain of M. marinum and one clinical isolate were investigated and, like M. tuberculosis, were found to have a single rRNA (rrn) operon per genome located downstream from murA gene and controlled by two promoters. No sequence differences were found that account for the difference in the growth rates of the two species. We infer that M. tuberculosis has the capacity to synthesize rRNA much faster than it actually does; and propose that the high number of insertion sequences in this species attenuate growth rate to lower values.—Authors’ Abstract


The Mycobacterium avium complex (MAC) encompasses two species, M. avium
and *Mycobacterium intracellularare*, which are opportunistic pathogens of humans and animals. The standard method of MAC strain differentiation is serotyping based on a variation in the antigenic glycopeptidolipid (GPL) composition. To elucidate the relationships among *M. avium* serotypes a phylogenetic analysis of 13 reference and clinical *M. avium* strains from 8 serotypes was performed using as markers two genomic regions (890 bp of the gtfB gene and 2150 bp spanning the rtfA-mtfC genes) which are associated with the strains’ serological properties. Strains belonging to three other known *M. avium* serotypes were not included in the phylogeny inference due to apparent lack of the marker sequences in their genomes, as revealed by PCR and Southern blot analysis. These studies suggest that serotypes prevalent in AIDS patients have multiple origins. In trees inferred from both markers, serotype 1 strains, known to have the simplest and shortest GPLs among all other serotypes, were polyphyletic. Likewise, comparisons of the inferred phylogenies with the molecular typing results imply that the existing tools used in epidemiological studies may be poor estimators of *M. avium* strain relatedness. Additionally, trees inferred from each marker had significantly incongruent topologies due to a well supported alternative placement of strain 2151, suggesting a complex evolutionary history of this genomic region.—Authors’ Abstract


We developed and evaluated a single-step, multiplex polymerase chain reaction (PCR) assay for distinguishing (1) between the *Mycobacterium tuberculosis* complex (MTBC) and mycobacteria other than tuberculosis (MOTT) and (2) between *M. tuberculosis* and *M. bovis* species. The assay targeted the 16S and the 23S rDNA to distinguish between MTBC and MOTT species, and the oxyR gene to distinguish between *M. tuberculosis* and *M. bovis* strains. Clinical samples and reference strains (N = 156) comprised 93 strains of *M. tuberculosis*, 44 of *M. bovis*, 1 *M. africanum* strain, and 18 strains representing 9 different species of MOTTs. MOTTs generated only a single PCR product of about 2.5 kilo-base; however, all of the MTBC strains produced a 118 base pair (bp) fragment and an additional 270 bp fragment was obtained for *M. tuberculosis* and *M. africanum* when the primer pair oxyRTB-2.1/oxyRMT-1 was used. When oxyRTB-2.1/oxyRMB-1 primers were used, the 270 bp fragment was obtained for only *M. bovis*. The assay needed as little as 1 pg of purified genomic DNA to make a positive identification.—Authors’ Abstract


See Current Literature, Microbiology, p. 384.


See Current Literature, Microbiology, p. 385.

**Sun, R., Converse, P. J., Ko, C., Tyagi, S., Morrison, N. E., and Bishai, W. R.** Mycobacterium tuberculosis ECF sigma factor sigC is required for lethality in mice
Bacterial alternative RNA polymerase sigma factors are key global adaptive response regulators with a likely role in Mycobacterium tuberculosis pathogenesis. We constructed a mutant lacking the sigma factor gene, sigC, by allelic exchange, in the virulent CDC1551 strain of M. tuberculosis and compared the resulting mutant with the isogenic wild-type strain and complemented mutant strain. In vitro, compared to the wild-type and complemented strains, the mutant was found to have similar ability to survive in both murine bone marrow-derived macrophages and activated J774 macrophages. In time-to-death experiments in the mouse model, the DeltasigC mutant was significantly attenuated, causing no death in infected mice whereas the wild-type and complemented strains caused 100% mortality within 235 days after aerosol infection with a median time to death of 170 days. Mouse organ bacterial burdens indicated that the mutant proliferated and persisted at the same level as the wild-type and complemented strains in lung tissue and was able to persist in mice without causing death for >300 days. A complete genomic microarray study demonstrated that SigC modulates the expression of several key virulence-associated genes including hspX, senX3 and mtrA, encoding the alpha-crystallin homologue, a two-component sensor kinase and a two-component response regulator respectively. Altered expression of a subset of these genes was confirmed by quantitative RT-PCR analysis. Analysis of genes modulated by SigC also revealed a putative consensus DNA recognition sequence for SigC of SSSAAT-N(16-20)-CGTSSS (S = C or G). Promoter recognition for one of these genes was confirmed by in vitro transcription analysis after purification of recombinant SigC and reconstitution of an ESigma(C) RNA polymerase holoenzyme. These data indicate that the M. tuberculosis transcription factor SigC governs expression of an important M. tuberculosis regulon and is essential for lethality in mice, but is not required for bacterial survival in this species. These observations place the DeltasigC mutant in a class of M. tuberculosis mutants which persist in tissues but are attenuated in their ability to elicit lethal immunopathology.—Authors’ Abstract


Infection with Mycobacterium tuberculosis causes the illness tuberculosis with an annual mortality of approximately 2 million. Understanding the nature of the host-pathogen interactions at different stages of tuberculosis is central to new strategies for developing chemotherapies and vaccines. Toward this end, we adapted microarray technology to analyze the change in gene expression profiles of M. tuberculosis during infection in mice. This protocol provides the transcription profile of genes expressed during the course of early tuberculosis in immune-competent (BALB/c) and severe combined immune-deficient (SCID) hosts in comparison with growth in medium. The microarray analysis revealed clusters of genes that changed their transcription levels exclusively in the lungs of BALB/c, SCID mice, or medium over time. We identified a set of genes (N = 67) activated only in BALB/c and not in SCID mice at 21 days after infection, a key point in the progression of tuberculosis. A subset of the lung-activated genes was previously identified as induced during mycobacterial survival in a macrophage cell line. Another group of in vivo-expressed genes may also define a previously unreported genomic island. In addition, our analysis suggests the similarity between mycobacterial transcriptional machinery during growth in SCID and in broth, which questions the validity of using the SCID model for assessing mycobacterial virulence. The in vivo expression-profiling technology presented should be applicable to any microbial model of infection.—Authors’ Abstract

To better understand genome function and evolution in Mycobacterium tuberculosis, the genomes of 100 epidemiologically well characterized clinical isolates were interrogated by DNA microarrays and sequencing. We identified 68 different large-sequence polymorphisms (comprising 186,137 bp, or 4.2% of the genome) that are present in H37Rv, but absent from one or more clinical isolates. A total of 224 genes (5.5%), including genes in all major functional categories, were found to be partially or completely deleted. Deletions are not distributed randomly throughout the genome but instead tend to be aggregated. The distinct deletions in some aggregations appear in closely related isolates, suggesting a genomically disruptive process specific to an individual mycobacterial lineage. Other genomic aggregations include distinct deletions that appear in phylogenetically unrelated isolates, suggesting that a genomic region is vulnerable throughout the species. Although the deletions identified here are evidently inessential to the causation of disease (they are found in active clinical cases), their frequency spectrum suggests that most are weakly deleterious to the pathogen. For some deletions, short-term evolutionary pressure due to the host immune system or antibiotics may favor the elimination of genes, whereas longer-term physiological requirements maintain the genes in the population.—Authors’ Abstract


The ability of Mycobacterium tuberculosis to grow in macrophages is central to its pathogenicity. We found previously that the widespread 210 strain of M. tuberculosis grew more rapidly than other strains in human macrophages. Because principal sigma factors influence virulence in some bacteria, we analysed mRNA expression of the principal sigma factor, sigA, in M. tuberculosis isolates during growth in human macrophages. Isolates of the 210 strain had higher sigA mRNA levels and higher intracellular growth rates, compared with other clinical strains and the laboratory strain H37Rv. SigA was also upregulated in the 210 isolate TB294 during growth in macrophages, compared with growth in broth. In contrast, H37Rv sigA mRNA levels did not change under these conditions. Overexpression of sigA enhanced growth of recombinant M. tuberculosis in macrophages and in lungs of mice after aerosol infection, whereas recombinant strains expressing antisense transcripts to sigA showed decreased growth in both models. In the presence of superoxide, sense sigA transformants showed greater resistance than vector controls, and the antisense sigA transformant did not grow. We conclude that M. tuberculosis sigA modulates the expression of genes that contribute to virulence, enhancing growth in human macrophages and during the early phases of pulmonary infection in vivo. This effect may be mediated in part by increased resistance to reactive oxygen intermediates.—Authors’ Abstract
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