Relapses in Multibacillary Patients Treated with Multi-drug Therapy until Smear Negativity: Findings after Twenty Years

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ABSTRACT
The Schieffelin Leprosy Research and Training Center at Karigiri, India participated in several of the World Health Organization (WHO) trials. The first trial on combined therapy in multi-bacillary leprosy was initiated in 1981. The main objectives of this field trial were to evaluate the efficacy of WHO recommended regimens in preventing relapses, especially drug resistance relapses. This paper reports on the relapses twenty years after patients were inducted into the WHO field trial.

Between 1981 and 1982, 1067 borderline lepromatous and lepromatous patients were inducted into the WHO field trial for combined therapy in multi-bacillary leprosy trial. Among them, 357 patients were skin smear positive. During the follow-up in 2002, only 173 of them could be traced and assessed. The mean duration of follow-up was 16.4 ± 1.83 years. Two patients relapsed 14 and 15 years after being released from treatment, the relapse rate being 0.07 per 100 person years follow-up. Drug susceptibility tests done on one of the relapsed patients revealed drug sensitive organisms to all multi-drug therapy drugs.

RÉSUMÉ
Le centre de recherche et de formation de Schieffelin à Karigiri aux Indes a participé à plusieurs études cliniques sponsorisées par l’Organisation Mondiale de la Santé (OMS). La première étude clinique sur la thérapie multiple contre la lèpre multi-bacillaire y fut initiée en 1981. L’objectif principal de cette étude de terrain était d’évaluer l’efficacité des prescriptions recommandées par l’OMS à prévenir les rechutes, en particulier les rechutes avec souches résistantes. Cet article rapporte les rechutes 20 ans après que les patients ont été enrôlés dans l’étude de terrain de l’OMS.

Entre 1981 et 1982, 1067 patients lépromateux et lépromateux borderline furent enrôlés dans l’étude clinique de terrain de polychimiothérapie au sein de l’étude de lèpre multibacillaire. Parmi ces derniers, 357 patients présentaient un résultat positif au test bactérioscopique du suc dermique. Durant le suivi de 2002, seuls 173 d’entre eux ont pu être retrouvés et évalués. La durée moyenne de suivi était de 16,4 ± 1,84 années. Deux patients ont
World Health Organization (WHO) field trials on Multi-drug therapy (MDT) regimens strongly recommend their use worldwide and are reported to be highly successful (4). Several studies have reported very low relapse rates after the completion of MDT, which must be interpreted with caution, as the duration of follow up in a majority of studies was relatively short (12). Relapses in leprosy following treatment with rifampicin-containing regimens are known to occur at least 5 ± 2 years after stopping treatment (15). As a consequence, it has been recommended that a follow-up of 10 years or more is required for drawing final conclusions on relapse rates after MDT (4, 11). The Schieffelin Leprosy Research and Training Center, Karigiri, was one of the centers to field test the WHO MDT regimens (Ref). An earlier paper reported no relapses after following up a subset of new, previously untreated patients for 13.7 ± 1.4 yrs. (16). This paper presents the latest data on relapses twenty years after they were inducted into the WHO field trial.

MATERIALS AND METHODS

The Schieffelin Leprosy Research and Training Center (SLRTC), Karigiri in India participated in several of the WHO MDT field trials, the first trial on combined therapy in multibacillary (MB) leprosy in December, 1981. The objectives of this field trial were to evaluate the efficacy of WHO recommended regimens in preventing relapses, especially drug resistant relapse. Of the 1067 multi-bacillary patients inducted into the trial during 1981 to 1983, only 357 patients were smear positive and were classified as either borderline lepromatous (BL) or lepromatous (LL) based on the Ridley-Jopling classification (1). According to their area of residence, patients were allocated to either of the two regimens being tested in the trial. Patients living in geographical divisions I and II were given Regimen A (THELEP) and those living in divisions III and IV were given Regimen B (Study Group). The regimens given are as follows:

Regimen A (THELEP regimen)

Rifampicin 600 mg on two consecutive days monthly—supervised + Clofazimine 600 mg on two consecutive days monthly—supervised +

Inj. Acedapsone (DADDS) 225 mg intramuscular once every two months + Dapsone 100 mg daily self-administered

Regimen B (Study Group regimen)

Rifampicin 600 mg once a month supervised +
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Clofazimine 300 mg once a month supervised +
Dapsone 100 mg once a month supervised +

Clofazimine 50 mg self-administered +
Dapsone 100 mg self-administered

The patients received the supervised dose at the clinic and compliance of the unsupervised drugs was monitored both by tablet count and the Dapsone:Creatinine ratio (D:C ratio) in urine. Smear positive patients were treated for a minimum period of 2 yrs or until smear negativity, whichever occurred later and then released from treatment (RFT). All patients recruited into the study were followed up annually with a clinical and bacteriological examination and a motor/sensory assessment. In 1995, only 225 of the original 357 smear positive patients could be followed up, after which annual follow-up and examination was discontinued.

In 2002, an attempt was made to follow-
up all living patients from the 357 smear positive patients belonging to the original 1982 cohort. All patients who could be traced underwent a clinical and bacteriological examination and a motor/sensory assessment. Any history of symptoms and signs of reactions were noted. For those patients who had died, a detailed verbal autopsy was done to establish the cause of death.

Relapse was diagnosed if two of the following three criteria were met: (i) occurrence of definite new skin lesion(s); (ii) increase of the BI of >2+ or over the previous value from any site; (iii) demonstration of viable \( M. \) leprae, by mouse footpad inoculation.

**RESULTS**

Of the 357 smear positive MB patients inducted into the trial in 1982, only 173 patients were available for follow-up in 2002 (vide flow chart). Eighty-nine patients belonged to Regimen A and 84 to Regimen B. The mean duration of follow-up of the 173 patients was 16.4 ± 1.83 yrs. Among those followed up, 75.1% were above 30 yrs of age, 70.5% were males, 61.3% were classified as LL and 12.1% had a BI of 3+ or more. Among the 184 patients not available for assessment, 101 (54.9%) had died, 65 (35.3%) had migrated and 18 (9.8%) were uncooperative. When compared to those not followed up, in the follow-up group there were significantly more males (81.5%; \( p = 0.02 \)), LL (72.3%; \( p = 0.035 \)) and patients with BI 3+ or more (45.7%; \( p <0.001 \)).

Among 173 patients assessed in 2002, the mean age was 57.2 yrs (S.D. = 10.4) (Table 1). There was no significant difference in the age and cause of death between patients treated with the two regimens. Since no significant differences were found in the characteristics of patients treated with the two regimens, they have been combined as one group for further analysis.

Table 2 shows the duration of treatment with dapsone monotherapy and MDT. The majority of patients had received dapsone monotherapy prior to MDT. The duration of dapsone monotherapy ranged between less than 8 weeks to more than 10 yrs. All patients were treated with MDT for a minimum of 2 yrs or till smear negativity. Seventy-eight patients (45%) had received MDT for 2 yrs, 90 (52%) for 3 to 5 yrs, and 5 (3%) for more than 5 yrs. The longest duration of treatment was for 2 patients who were given MDT for 8 yrs. It is observed that there is no significant association between the duration of monotherapy received prior to MDT and the duration of MDT subsequently required by patients to reach smear negativity (\( p = 0.13 \)).

Among the 173 patients assessed in 2002, two patients were diagnosed as relapse; a relapse rate of 0.07 per 100 person years follow-up. Both patients were males, who had Dapsone monotherapy prior to MDT and had received Regimen B. The first patient relapsed 15 yrs after RFT. He was diagnosed as LL in 1976 and was initiated on monotherapy, which he took regularly until 1982. In 1982, he was inducted into the WHO MDT trial. His initial bacterial index (BI) in 1976 was 3.50+ and his BI just prior to initiation of MDT had come down to 2.0+. Subsequent to treatment with MDT, he became negative after 4 yrs of MDT in 1986. He was RFT in 1987 and was followed up annually for 9 yrs, until 1996. He remained negative till then. During the follow-up in 2002, he was found to have new skin lesions that showed signs of activity and on smear examination had an average BI = 1.3+ at routine sites and 4.0+ at selective sites. He was diagnosed as...
relapse and restarted on MDT. The mouse footpad inoculation detected viable bacilli in T900r mice and they were sensitive to all MDT drugs.

The second patient relapsed 14 yrs after RFT. He was diagnosed as LL in 1967 when he was 9 years old, and was initiated on Dapsone monotherapy, which he took regularly for 15 yrs. His initial BI prior to monotherapy was 3.25, which came down to 0.8 in 1982, when he was inducted into the WHO MDT trial. Two years after MDT, he became smear negative in 1984 and was RFT. Annual assessments were done for 11 yrs after RFT until 1995. In 1998, he voluntarily presented with active skin lesions and a smear examination revealed an average BI of 1.0+ in routine sites and 5.0+ at selective sites. Mouse footpad inoculation was not done on this patient. He was diagnosed as relapsed and restarted on MDT. He became smear negative after 2 yrs.

**DISCUSSION**

As in the case of other infectious diseases, the relapse rate is a crucial parameter in assessing the long-term efficacy of chemotherapy. The relapse rate after WHO recommended MDT regimens is generally accepted to be low. A WHO questionnaire survey reported that the cumulative risk of relapse was 0.77% for multi-bacillary (MB) leprosy patients, 9 yrs after stopping MDT (22). Other follow-up studies have reported relapse rates varying from less than 1% to 20.0% (5, 12, 15). The AMFES study reported no relapses after a mean duration of follow-up of 5 yrs (9), and a more recent paper reported no relapses after a follow-up of 13 yrs. (18).

In this study, 173 smear positive patients were followed up, twenty years after they were initiated on MDT. The mean duration of follow-up was 16.4 ± 1.83 yrs after RFT, which perhaps is the longest follow-up of a cohort of MB patients reported. The relapse rate in this study is 0.07 per 100 person years follow-up, which is lower than the acceptable relapse rate of 1.0 per 100 person years and relapse rates reported in other studies (15). Nevertheless, three important aspects need to be considered while interpreting these findings. Firstly, the two patients who relapsed had 6 and 15 yrs of monotherapy prior to being initiated on MDT. Secondly, patients in this trial were treated for a minimum period of 2 yrs or until smear negativity, whichever occurred later. Thirdly, the patients not followed up, were significantly different from those followed up, in terms of more males, LL and higher BI at induction. Thus this cohort may not represent the untreated new patients currently being treated with WHO-MDT recommended regimens.

It is known that the rate of relapse is governed by two factors; namely, the high initial bacterial load and the long period of follow-up (7). The initial bacterial load before and at RFT is noted to be closely correlated to the risk of relapse. Among patients with an initial BI of ≥4.00+, the relapse rate was reportedly 38.9% as compared to no relapses among those with an average BI of <4.00+ (12). In another study of patients treated up till smear negativity, a higher relapse rate of 1.27 per 100 person years was observed among patients with a initial BI of ≥4.00+ as compared to 0.46 per 100 person years among patients with a initial BI of <4.00+ (10). In the present study, patients who relapsed had a high initial BI of ≥4.00+ prior to monotherapy. They continued to be positive with BI ≥ 0.8+ and 2.0+ when they were recruited into the trial. In contrast, only a few relapses have been reported among patients with a high initial bacterial index treated with fixed duration therapy (FDT) (9, 14, 17). However, the low relapse rates among these patients with a high bacterial index

### Table 3. Relapses.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sex</th>
<th>Initial type</th>
<th>Initial BI</th>
<th>Duration of mono</th>
<th>Duration MDT</th>
<th>Time of relapse after RFT</th>
<th>BI at relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>LL</td>
<td>3.50</td>
<td>6</td>
<td>5</td>
<td>15 years</td>
<td>Routine 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Selective 4.0</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>LL</td>
<td>3.25</td>
<td>15</td>
<td>2</td>
<td>14 years</td>
<td>Routine 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Selective 5.0</td>
</tr>
</tbody>
</table>
could be attributed to the relatively short duration of follow-up.

Relapses after treatment with rifampicin containing regimens are said to occur late, usually more than 5 yrs after RFT \(^{8,12,15,19}\), and therefore it is considered necessary that patients should be followed for a minimum of 7 to 10 yrs after completion of treatment \(^{4,8}\). This contrasts with information from certain authors that 75% of relapses occurred during the first 4 yrs after completion of treatment and also that annual risk of relapse does not increase with time \(^{6}\).

The patients who relapsed in this cohort had a long duration of follow-up after MDT before being diagnosed as having a relapse. An earlier paper reported no relapses after following a subset of patients from the same cohort for 13.7 ± 1.4 yrs \(^{16}\). Both relapses reported in the present study had dapsone monotherapy prior to MDT and hence were not reported in the above paper. Relapses may be either due to persisters or reinfection \(^{19}\). In the absence of any method to prove reinfection, it is reasonable to assume that both the relapses reported are due to persisters; however, reinfection in these patients cannot be ruled out.

Relapse was diagnosed in both the patients 15 and 14 yrs after RFT underlining the importance of continued surveillance after RFT, at least in those patients with an initial high bacillary index. However, in spite of a field program wherein long-term surveillance after RFT was possible, more than 50% of the initial cohort was lost to follow-up due to deaths and migration. Thus long-term follow-up may not be cost-effective or feasible and education of the patient to report soon after appearance of new skin lesions may perhaps be the best option in the integrated setting.

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Design of the Leprosy Component of the Brazilian BCG Revaccination Trial for Assessing BCG Effectiveness against Leprosy in School Children

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ABSTRACT

Background. BCG vaccination confers protection against leprosy, and vaccination among household contacts has been recommended in Brazil. Nevertheless, vaccination of the entire community against leprosy is not advocated as leprosy has low incidence in most populations. Despite that, in Brazil, BCG vaccination is recommended among school children to prevent tuberculosis and this large scale vaccination may also affect the occurrence of leprosy, which led to investigations of its impact on leprosy in endemic areas of Brazil.

Objectives. To estimate the effectiveness against leprosy of a dose of BCG vaccine given to school children in a population with a high coverage of neonatal BCG. Long term objectives are to compare the impact of vaccination among schoolchildren with the existing recommendation to vaccinate household contacts of leprosy.

Study design. Cluster randomized controlled field trial with no placebo.

Study population. Children aged 7 to 14 years attending state schools with high coverage of neonatal BCG.

Methods. 286 state schools in the city of Manaus, Brazil, were randomized to receive BCG or not. Identifying information was collected for 152,438 school children, of whom 72,980 are in intervention schools. BCG vaccination was given intradermically to children in schools allocated to vaccination. Follow-up relies on ascertainment of cases diagnosed at the health services and notified to the reference center for leprosy.

RÉSUMÉ

Background. La vaccination par le BCG confère une protection contre la lèpre, et la vaccination des contacts vivant sous le même toit a été recommandée au Brésil. Cependant, la vaccination de tous les membres d’une communauté contre la lèpre n’est pas encouragée parce que la lèpre présente une incidence faible dans la plupart des populations. Malgré cela, la vaccination par le BCG est recommandée au Brésil pour les enfants en âge d’aller à l’école pour la prévention de la tuberculose et cette vaccination à grande échelle pourrait aussi influencer l’ incidence de la lèpre, ce qui nous a amené à explorer son impact éventuel sur la lèpre dans les régions endémiques du Brésil.

Objectifs. Estimer l’efficacité contre la lèpre d’une dose de vaccin BCG administré à des enfants scolarisés dans une population présentant une forte couverture de vaccination au BCG à la naissance. Les objectifs à long terme sont de comparer l’impact de la vaccination...
It is well established that a single dose of BCG confers protection against leprosy, with estimates ranging from 20% to 90% efficacy in different studies (8-12), and multiple doses of BCG appear to confer additional protection (3, 5, 11) (although this finding has not achieved statistical significance in the study in Venezuela) (5). However, as leprosy has low incidence in most populations, the number of individuals needed to be vaccinated to prevent one case of leprosy tends to be large (20) unless applied in high risk groups. Therefore, the Ministry of Health in Brazil recommends vaccination only among household contacts of leprosy cases (16).

Brazil has had a high coverage of neonatal BCG since the 1980’s. In 1994 the Brazilian Ministry of Health recommended the routine application of BCG among school children to prevent tuberculosis (17), and a trial (BCG-REVAC) was proposed to assess the effectiveness of the recommended measure. This trial is being conducted in two cities, Salvador and Manaus (2). Brazil, with a population of nearly 170 million, has the second largest number of leprosy cases in the world (23). Vaccine efficacy trials aim to estimate the protection under ideal conditions (4), and are standard for vaccine licensure by regulatory agencies. However, the results of such studies do not correspond to the protection given by the vaccine in the target population under routine conditions. The effectiveness trial concept was proposed to incorporate the randomization process and elements of a public intervention implementation approximating routine conditions. Effectiveness trials attempt...
to reproduce real conditions. The BCG-REVAC trial was planned as a vaccine effectiveness trial, and its study design attempts to reproduce the routine implementation of a BCG vaccination policy to school children according to the 1994 recommendation. Manaus, one of the trial sites, is an endemic area of leprosy. This trial created the opportunity to estimate the impact of the recommended BCG vaccination among school children on the occurrence of leprosy. We therefore expanded the objectives of the trial in the city of Manaus—where the recommendation to vaccinate contacts has not been completely implemented so far—to include the estimation of protection of BCG against leprosy.

The objective of this paper is to describe the rationale, study design, implementation and proposed analysis of the leprosy component of the trial. The design of the trial component for preventing tuberculosis has been published elsewhere (2).

**DESIGN AND METHODS**

The main objective of the leprosy component of the trial is to estimate the protection against all forms of leprosy of one dose of BCG given under routine conditions. Long-term objectives are to estimate effectiveness by age and clinical form, and to compare this policy with the existing recommendation to vaccinate household contacts of leprosy, in terms of impact on occurrence of leprosy and in term of costs. This text will only describe the main objective.

**Study site.** Manaus is an urban center on the banks of the Negro River, Amazonas State, in Brazil, with about 1,500,000 inhabitants in 2002. The annual new case detection rate (NCDR) of leprosy has been stable at around 6.0 per 10,000 inhabitants in the 1990’s. In 1997 it was 6.6 (814 cases) in the total population and 4.9 (110 cases) in children aged 7 to 14 yrs. Sixty percent of all cases detected in 1996 were classified as tuberculoid or indeterminate and 40% as multibacillary.

**Study population.** The target population consisted of school children residing in Manaus, as that covered by the 1994 recommendation for BCG vaccination against tuberculosis, and restricted to those aged 7 to 14 yrs and attending state schools at the time of the trial implementation (born between 1984 and 1991). Only schools with more than 50 pupils in this age group were included in this study.

This population has a high coverage of neonatal BCG: official data reported coverage rate of 56.8%, 74.8%, 82.0%, 89.0%, for those born in 1988, 1989, 1990, and 1991, respectively. Therefore, for most people this will be a second dose. No child was excluded on the basis of previous history of tuberculosis or leprosy.

**Screening for detecting leprosy and HIV infection.** An increase in the risk of leprosy has been described in the initial follow-up period in some trials (10, 13) (but not reported in all of them). However, this trial did not include screening for leprosy before vaccination. This was for two reasons. First, the trial aims to estimate the vaccine effectiveness under the recommended policy for tuberculosis and this policy does not include previous screening (17), even though Brazilian policy for BCG vaccination among household contact of leprosy includes screening for detecting leprosy before vaccination. Such a screening among school children as a requirement to vaccination would probably not be feasible because of the large number of individuals, and would be a time-consuming operation. Secondly, the increase in risk for leprosy, if present, was expected to be transient and would not be considered an obstacle to a BCG large-scale vaccination in leprosy endemic areas.

Although BCG vaccination carries a risk of severe adverse effects when given to people with AIDS (22), screening for HIV infection was not done. The prevalence of AIDS in Manaus and in this age group is low (one case was reported during the whole period of 1998 to 2001 among those aged 7 to 14 yrs) (15), and the 1994 recommendation for BCG among school children does not require HIV screening.

**Outcomes.** All leprosy cases from Manaus reported by the local surveillance service and belonging to the target population have been recorded. The incidence of leprosy is expressed as the NCDR of leprosy per 10,000 person years in the two comparison groups during the trial follow-up.

**Sample sizes and follow-up period.** Leprosy has a long incubation period (7) but the protective effect of BCG against leprosy was observed very soon in some trials (1, 21).
As most cases in Manaus are paucibacillary, with a shorter incubation period (7), the sample size was chosen to allow a first intermediate analysis after 4 to 5 yrs of follow-up. The study power at this phase would not necessarily be sufficient for detection of differences in VE between clinical forms or previous vaccination status. To estimate the sample size for the leprosy component of the trial, we used the formula described in chapter 7 in Friedman, et al. (9):

\[
2N = 2\left[\frac{Z_\alpha \sqrt{2P(1 - P) + Z_\beta \sqrt{Pc(1 - Pc) + Pi(1 - Pi)}}}{\sqrt{Pc - Pi}}\right]^2/(Pc - Pi)^2
\]

Where Pc and Pi are the incidence proportions in control and intervention groups, respectively; \(P = (Pc + Pi)/2\), \(Z_\alpha\) is the critical value corresponding to the significance level \(\alpha\) type I error; \(Z_\beta\) corresponds to the power. The following parameters were used to estimate the sample size:

- study power of 80% and 90%;
- type I error of 5%;
- VE of 50% [as observed in the Malawi trial (11)];
- NCDR of 4 per 10,000 per year in the control group (overall NCDR in this age group in Manaus),
- and assuming that rate is constant with age over time.

This resulted in estimates for different follow-up periods and study power, presented in The Table. Finally, it was agreed on a sample size of 50,000 individuals in each arm. This number was below the sample size of the original tuberculosis trial for the city of Manaus (7). Leprosy cases reported before the trial started, but who would have met the trial entry criteria, were used to estimate the intra-class correlation (ICC) and the design effect (DEF) of the NCDR of leprosy. The ICC was estimated taking schools as clusters. ICC and DEF were estimated as -0.00088 and 0.95, respectively, and thus the sample size was not inflated.

**Randomization.** Schools (\(N = 286\)) were the randomization unit and were firstly allocated to strata defined on the basis of number of students registered in 1996 in the age group, and incidence of leprosy and tuberculosis in 1996 in the geographical areas where schools were located. The city of Manaus is divided in 56 administrative areas (districts) that are grouped into 6 greater geographical areas (South, North, West, East, Center-West, Center-South). These 56 districts were categorized into those with incidence of leprosy (NCDR) below or above the incidence of the city as a whole. The same procedure was used for tuberculosis. The districts were then classified into five levels: four combinations of incidence of tuberculosis and leprosy, and a fifth category of the districts with unreliable or unavailable data on tuberculosis or leprosy. The 286 schools were then sorted by geographical areas, number of students and incidence of leprosy and tuberculosis, and paired. One school in each pair was allocated at random to the control group—the other was allocated to the intervention group. When a school had no pair (odd number of schools in the stratum), this last school was allocated at random.

**Implementation.** Trial coordination. Overall co-ordination of the trial is from the Instituto de Saúde Coletiva (Brazil) and London School of Hygiene and Tropical Medicine (U.K.). Collaborating centers are: Fundação Alfredo da Matta (FUAM), Brazilian National Health Foundation and Brazilian National Immunisation Programme. The planning and co-ordinating research team for Manaus included the local co-ordinators and most members of the team of the second study site of the tuberculosis component (Salvador). There were two local co-ordinators: a clerk with experience in implementing surveys was responsible for the recruitment and data collection, and a nurse with experience in managing surveillance and BCG vaccination was re-

**THE TABLE.** Size in each arm needed for an observed vaccine effectiveness of 50% in Manaus and for study power of 80% and 90%.

<table>
<thead>
<tr>
<th>Year of follow-up</th>
<th>Cumulative incidence (%)</th>
<th>Study power</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>80%</td>
</tr>
<tr>
<td>1st</td>
<td>0.04</td>
<td>117,563</td>
</tr>
<tr>
<td>2nd</td>
<td>0.08</td>
<td>58,764</td>
</tr>
<tr>
<td>3rd</td>
<td>0.12</td>
<td>39,164</td>
</tr>
<tr>
<td>4th</td>
<td>0.16</td>
<td>29,364</td>
</tr>
</tbody>
</table>

*Baseline incidence estimated in 4 per 10,000 a year (0.04%).
responsible for vaccination and surveillance for adverse effects. Professionals from the local leprosy reference service (Fundação Alfredo da Matta, FUAM) are involved in the ascertainment and validation of leprosy cases.

**Recruitment, vaccine ascertainment, and vaccination.** In the recruitment phase, field workers transcribed data (full name, date of birth, sex, full name of the mother, address) from school records to a standard form. This was done without contact with the students. Afterwards, BCG scar was read to ascertain neonatal vaccination status (2).

Vaccination (0.1 ml) was done by trained nurses through intradermal injection in the deltoid region of the right arm. The lyophilized BCG used in Brazil contains the Moreau strain, related to the Japanese and Russian strains (6). This is the vaccine used by the Brazilian National Immunization Program (PNI), and has been shown to offer high protection against leprosy in Brazil (12, 14, 19). Four different batches were used in Manaus and vaccination in each school was done with a single batch. The vaccines were stored at the local immunization program and delivered to the campaign headquarters when required. The vaccines were kept refrigerated and the temperature checked regularly using the routine cold chain facilities.

**Surveillance of adverse events.** The routine passive surveillance for adverse events was enhanced. A letter containing information on BCG adverse events was distributed to all children on the day of vaccination to motivate parents to take children to a health facility if they had a health problem following vaccination. Health workers in the reference medical centers for tuberculosis and leprosy were made aware of the trial and alerted to possible BCG adverse events. Suspected adverse events were expected to be diagnosed in the health facilities and treatments for adverse events provided. This vaccine safety surveillance continued for 4 months after the end of vaccination.

**Avoidance of bias.** This study is not a double-blind trial and there was no concealment of allocation, so study participants knew whether they received the vaccine, which could lead to detection bias. However, bias is not expected as those with clinical features of leprosy are equally likely to seek medical attention irrespective of vaccination status. Linkage of leprosy cases to the study population is done blind to vaccine status, and physicians are not aware about the BCG status of the most leprosy cases (see below).

Another potential source of contamination is vaccination in individuals allocated to the control group after the start of the trial due to vaccination among household contacts of leprosy cases, leading to an underestimate of the vaccine effectiveness. However, this vaccination in contacts is scarcely implemented in Manaus (in 1998, official data reported 60 individuals for all age groups who received BCG to prevent leprosy) (15) and the managers of the local immunization program agreed in not enhancing this vaccination among contacts before the first phase of the trial.

**Follow-up procedures.** Passive follow-up and case ascertainment. Follow-up consists of identifying cases in the surveillance system and establishing whether they are from the trial population through linkage. Although several health facilities undertake leprosy diagnosis in Manaus, 70% of all cases are diagnosed and treated in the local leprosy reference center (FUAM), where the state epidemiological surveillance service for leprosy is based. All leprosy cases in FUAM are submitted to biopsy and skin smear. A standardized notification form is routinely completed for all leprosy cases in the other diagnosing units and forwarded to FUAM where data are entered into a computerized database, so data from all leprosy cases can be easily obtained.

Physicians are asked to refrain from inquiring about the BCG status until a definitive diagnosis is made, unless the physician judges it necessary to know the BCG status for good clinical practice. A standard form was developed in the local reference center (FUAM) to collect information on whether the clinician was aware of the BCG vaccination status of the patient. Cases are routinely categorized according to the criteria adopted by the Brazilian Ministry of Health (based on the Madrid Conference and two groups system proposed by the World Health Organization) (16), and based on Ridley-Jopling classification (18).

**Setting up the database and linkage of cases.** The study has two databases. The data on the recruited study population were
entered in the first database (study population database). It contains identification data (which were transcribed from school records to a standardized form during the recruitment phase), neonatal BCG vaccination (mainly from BCG scar reading) and vaccination by the trial. Clinical and laboratory data for each case of leprosy detected during the follow-up, as recorded in the reference center and by the surveillance services, are entered in a second database (case database). Records in the case database are matched to the records in the study population database, based on variables present in both databases: subject’s name, date of birth, sex, and mother’s name, without access to data on whether the study participants had received BCG. This matching is done twice by two independent researchers.

**Concerns.** This trial as a whole is a joint collaboration involving the two academic institutions and the Brazilian Ministry of Health. Two ethical committees (Universidade Federal da Bahia, Brazil; and London School of Hygiene and Tropical Medicine, U.K.) approved the original trial of tuberculosis. The leprosy component trial was added to the trial of tuberculosis later. The initial view was that ethical approval was not required because the main additional activity was the ascertainment of leprosy cases from routine sources. This was revised later on, and the trial received ethical committee approval by the National Ethical Committee in 2003.

**RESULTS**

Field work (recruitment) in Manaus began in July and finished in December 1998. Vaccination began in September and finished in December 1998. The starting point for the follow-up of new leprosy cases was on 1 January 1999.

The Figure shows the population as enrolled in the study and the sub-populations to be used in the estimate of the VE. Records were collected in 286 schools (140 schools in intervention group and 146 in
control group). Information on identification was collected on 156,331 individuals, corresponding to about 69.0% of the population of Manaus in this age group. From this group, 3893 individuals were excluded because they were outside the target age group and thus 152,438 individuals remained: 79,458 in schools allocated to the control arm and 72,980 in schools allocated to vaccination. The proportion of individuals with a BCG scar reading was similar in control and intervention groups (79.9% vs 76.3%, respectively). The groups in which the VE will be estimated are constituted of 110,218 individuals with either no BCG scar or one BCG scar. Among those 51,207 in the intervention group, 46,997 were vaccinated and 4210 were not due to the following reasons: 11 had already two BCG doses reported in vaccination cards or by the guardians, 843 refusals by the school children, 2342 refusals by their guardians, 68 were not present at the moment of vaccination, 946 for reasons not documented.

**DISCUSSION**

VE will be estimated by comparing the incidence of leprosy among individuals in the original allocation groups, but including only those with BCG scar reading and of these, only those with no scar or one BCG scar (N = 110,218). The first analysis will be done when the total number of leprosy patients in this group allows a study power above of 80% for a vaccine effectiveness of 50%. This analysis consists of an intention-to-treat approach in which 92% (46,997) of the individuals allocated to vaccination were vaccinated. So, a vaccine effectiveness of 50% will be observed as 46%, which does not represent a significant loss of power.

The proposed plan of analysis for the main objective will mainly consist of three phases. First, the description of the baseline characteristics of the study population. Second, the effect of cluster will be estimated. Third, estimation of the VE will be obtained as the percent reduction in NCDR per person-year among unvaccinated individuals as compared to those vaccinated. The VE will be estimated for the total number of leprosy cases and separately for time period from vaccination, age group, sex, neonatal BCG vaccination and clinical forms. The estimation of VE will be estimated by standard statistical procedures and also adjusted by the effect of clustering.

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**Contributions.** All authors fulfilled the criteria for authorship proposed by Vancouver Conference: concept of the study, drafting the article or revising it critically for important intellectual content and all had the final approval for its content. S. C. Cunha, L. C. Rodrigues, and M. L. Barreto had the primary responsibility for epidemiological study design. E. S. Pereira, M. F. Maroja, and C. Ribas (Fundação Alfredo da Matta) are engaged in the case detection. Mr. José Carlos and Ms. Fátima Praia were primarily responsible for the recruitment and vaccination.

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IL-10 Treatment of Macrophages Bolsters Intracellular Survival of *Mycobacterium leprae*1, 2

Yasuo Fukutomi, Masanori Matsuoka, Fumishige Minagawa, Satoshi Toratani, Gregory McCormick, and James Krahenbuhl3

**ABSTRACT**

In these studies, metabolically active *Mycobacterium leprae* were maintained for as long as 8 weeks in monolayer cultures of mouse peritoneal macrophages (MΦ). Supplemental IL-10, but not TGF-β, bolstered, directly or indirectly, *M. leprae* metabolism in mouse MΦ. In the cell culture system temperature setting is extremely important and 31 to 33°C incubation temperature was more permissive than 37°C. Acid fast staining and transmission electron microscopy (TEM) of intracellular *M. leprae* revealed visible elongation of bacilli cultured under the above ideal conditions.

**RÉSUMÉ**

*Mycobacterium leprae* n’a jamais encore été vraiment cultivé sur milieu artificiel. Comme *M. leprae* préfère vivre in vivo à l’intérieur de la cellule, nous avons exploré la croissance in vitro de *M. leprae* dans des cultures de phagocytes mononucléés ou de macrophages, qui représentent la cellule hôte favorite du bacille de la lèpre. Notre approche expérimentale a tenu compte des faiblesses de ce type d’expérimentation : à savoir la viabilité de l’inoculum et la longue durée de multiplication de *M. leprae*. Le but n’était pas ici de démontrer une augmentation mesurable et significative du nombre de *M. leprae*, mais plutôt le maintien prolongé du métabolisme de *M. leprae*, en mesurant l’oxydation de l’acide palmitique radiomarqué, comme indicateur de viabilité.

Des *M. leprae* ayant un métabolisme actif ont été maintenues jusqu’à 8 semaines dans des cultures mono-couches de macrophages péritonéaux de souris. L’ajout d’IL-10, mais pas de TGF, a augmenté, directement ou indirectement, le métabolisme de *M. leprae* dans les macrophages de souris. Le réglage de la température des cultures cellulaires est extrêmement important et une température d’incubation de 31–33°C était plus favorable qu’une température de 37°C. La coloration acido-alcoolo-résistante et la microscopie électronique à transmission des *M. leprae* a permis de mettre en évidence des elongations bien visibles parmi les bacilles cultivés dans les conditions idéales mentionnées ci-dessus.

**RESUMEN**

*Mycobacterium leprae* todavía no se ha podido cultivar en medios artificiales. Como esta bacteria prefiere una existencia intracellular in vivo, en este estudio exploramos el crecimiento in vitro de *M. leprae* en cultivos de fagocitos o macrófagos, el huésped preferido del bacilo de la lepra. Reconocemos que nuestro diseño experimental conlleva dos problemas: la viabilidad de *M. leprae* en el inóculo, usualmente baja, y el prolongado tiempo de división de la bacteria. Aunque no esperábamos encontrar un incremento sustancial en los números de *M. leprae*, sí pensamos poder observar cambios en su metabolismo midiendo la oxidación del ácido palmitico radiactivo como un marcador de viabilidad. Encontramos que la bacteria se mantuvo metabólicamente activa hasta por 8 semanas en los cultivos de los macrófagos peritoneales de ratón. La adición de IL-10 pero no de TGF, apoyó, directa- o indirectamente el metabolismo de *M. leprae* en los macrófagos de ratón. Las condiciones de incubación de los cultivos fueron muy importantes y la temperatura de 31–33°C fue más permissiva que la temperatura de 37°C. La tinción para ácido-resistentes y la microscopia electrónica de transmisión revelaron cierto grado de alargamiento de los bacilos bajo las condiciones óptimas de cultivo de los macrófagos.
In the 130 years since the discovery of *Mycobacterium leprae* as the causative agent of leprosy, a large number of attempts have been made to cultivate this obligate intracellular pathogen in cell-free media (6, 30). None of these efforts have fulfilled the criteria for success suggested by John Hanks (18), especially the confirmation of findings by a second laboratory. The inability to culture the leprosy bacillus has undoubtedly hindered almost every aspect of leprosy research, and thus, our understanding of this disease lags behind that of many others of bacterial etiology.

Reasoning that the intracellular milieu would best suit the multiplication of the obligate intracellular leprosy bacillus, as an alternative to culture in axenic medium, a number of attempts have been made to cultivate *M. leprae* in various types of cultured cells. Fieldsteel and McIntosh (10) employed a range of rat, mouse, and human tissue but found no evidence of multiplication. The preferred host cell for the leprosy bacillus, however, appears to be the mono-nuclear phagocyte or macrophage (MΦ) and a number of unsuccessful attempts have been made to grow *M. leprae* in MΦ (5, 8, 16, 24, 27, 31, 34, 36, 41). The present approach also employs MΦ but is novel in that we employed conditions that inhibit innate antimicrobial functions in infected mouse MΦs to bolster the intracellular survival of *M. leprae*. Moreover, we had a number of advantages over the previous attempts by others including: unique resources, previously unavailable to other workers in the form of fresh, highly viable *M. leprae* (25), sensitive techniques for measuring and comparing the metabolic activity of *M. leprae* (13) and the extensive experience of our two laboratories in studying the relationship between the MΦ and the leprosy bacillus (2, 14, 15, 39, 40).

**MATERIALS AND METHODS**

**Maintenance of a viable *M. leprae* inoculum.** The Thai-53 strain of *M. leprae* (26) was maintained in continuous passage in athymic nu/nu mice (Crea Co., Tokyo, Japan) by inoculation of 1 × 10⁶ freshly harvested bacilli into both hind footpads. At approximately nine months post, footpads were processed to recover *M. leprae* by Nakamura’s method with a slight modification (28). Briefly, tissue was minced and homogenized with Hanks’ balanced salt solution (HBSS) containing 0.05% Tween 80. The homogenate was centrifuged at 150 × g for 10 min and supernatant of the sample homogenate was treated with 0.05% trypsin at 37°C for 60 min. The suspension was centrifuged at 4000 × g for 20 min and sediment was re-suspended in HBSS followed by treatment with 1% sodium hydroxide at 37°C for 15 min. The treated material was washed and re-suspended in HBSS at the desired bacillary concentration. Bacillary number in each footpad was enumerated individually according to standard techniques (37).

**Cytokines.** Murine recombinant IL-10 was obtained from Genzyme Corp. T cell growth factor β (TGF-β) was obtained from Kurashiki Bouseki, Kurashiki, Japan. Both cytokines were stored at −80°C until use.

**Mouse MΦ culture.** Mouse peritoneal resident cells (approximately 50% MΦ) were harvested from retired ICR or Swiss White (SW) mice and suspended as previously described (2) at a concentration of 2 × 10⁶/ml in RPMI 1640 (GIBCO, Grand Island, NY) + 15% fetal bovine serum (HyClone Laboratories, Logan, UT) + 25 mM hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) (GIBCO), 0.2% NaHCO₃ (GIBCO), 2 mM glutamine (Irvine Scientific, Santa Ana, CA), and 100 µg/ml ampicillin (Sigma Chemical Co., St. Louis, MO). 0.5 ml was seeded into 24 well tissue culture plates (Corning) containing 16 mm LUX coverslips (Miles Laboratory, Naperville, IL). After overnight adherence of the cells, MΦ monolayers were obtained after washing non-adherent cells from the coverslip with Hanks Balanced Salt Solution (HBSS) leaving approximately 1 × 10⁶ MΦ adhered per coverslip.

**Infection of MΦ with *M. leprae*.** Purified mouse MΦ monolayers were infected with fresh *M. leprae* suspended in 0.5 ml medium at a multiplicity of infection of 20:1. After 4 hr incubation, non-phagocytized bacteria were removed by washing and the cultures reincubated in 1.0 ml media supplemented with the appropriate cytokine in 5% CO₂ at the appropriate experimental temperatures (°). Media was changed and, where appropriate, cytokines replenished at 5 day intervals.

**Radiorespirometry (RR).** The MΦ were lysed with 0.1 N NaOH to release the *M.
leprae, and the viability of the bacilli was determined by evaluating the oxidation of $^{14}$C-palmitic acid to $^{14}$CO$_2$ by radiorespirometry as described previously (13). Total isotope release was usually analyzed after one week of incubation at $31^\circ$C (2).

Staining of M. leprae-infected MΦ. Coverslips of M. leprae-infected adherent MΦs were prefixed with absolute methanol, and acid-fast stained Photomicrographs were taken using a Nikon Optiphot microscope using an oil immersion Plan APO 100 lens.

Transmission electron microscopy (TEM). MΦ monolayers on coverslips were pre-fixed in 2% glutaraldehyde/0.1 M Na-cacodylate buffer followed by postfixation with osmium tetroxide/K-CN and endoblock staining with uranyl acetate. The
FIG. 2A. Metabolic activity of *M. leprae* in MΦ cultured in the presence of IL-10 or TGF-β. *M. leprae* infected MΦ were incubated at 31°C in the presence or absence of 2 U/ml IL-10 or 10 ng/ml TGF-β and bacilli were released from infected MΦ on the days shown (in triplicate) and inoculated into RR vials. The data shown represent RR data obtained after 7 days.

Fig. 2B. Comparison of metabolic activity of *M. leprae* in MΦ cultured at 31°C and 37°C in the presence of IL-10. *M. leprae* infected MΦ were incubated at 31°C or 37°C in the presence or absence of 2 U/ml IL-10 and bacilli were released from infected MΦ on the days shown (in triplicate) and inoculated into RR vials. The data shown represent RR data obtained after 7 days.

RESULTS

In vitro temperature preferences of *M. leprae*. *M. leprae* clearly prefers cooler incubation temperatures. As shown in Figure...
In axenic culture, it was apparent that 37°C was not an ideal temperature to demonstrate sustained viability. Incubation at 35°C was more supportive than 37°C, and results at 29°C and 32°C were indistinguishable but even more ideal.

Similarly, intracellular *M. leprae* thrives better at cooler temperatures. Mouse MΦ appeared to function normally at 33°C and even 31°C, as judged by attachment to plastic and phagocytic capacity, although they did not spread as well at these lower temperatures as they do at 37°C. In the experiment depicted in Figure 1B, infected MΦs were incubated at either 31°C or 37°C and at 5 day intervals released bacilli were studied by RR for an additional 7 days. The detrimental effects of incubation at 37°C on *M. leprae* metabolism were apparent by day 5. In marked contrast, *M. leprae* cultured in MΦ at 31°C thrived for at least 15 days and retained most of its viability after 25 days in MΦ maintained at the lower temperature.

**Effects of cytokines on viability of *M. leprae* in MΦ.** Supplementation of the infected MΦ culture medium with 2 U/ml murine IL-10 was clearly associated with sustained viability of intracellular *M. leprae*. In the more prolonged experiments depicted in Figure 2A and 2B, *M. leprae* steadily lost viability in control MΦ at 31°C and 37°C. In contrast, in MΦ incubated in the presence of IL-10, *M. leprae* maintained their viability, but only at the permissive temperature of 31°C. (Figure 2A and 2B). As shown in Figure 2A, addition of TGF-β to the infected MΦ had no effect on the viability of *M. leprae*.

Experiments were run to account for all of the *M. leprae* in the long term cultures, assuming that during prolonged culture some infected MΦ may detach or lyse, releasing their bacilli. In the experiment depicted in Fig. 3, media was changed as usual every 5 days and data points recorded every 10 days. In order to account for bacilli released from MΦ or bacilli in “detached” MΦ we collected and saved the “old” media at 4°C at the time it was changed (midpoints of the 10 day time points plotted at 20, 30, 40 days, etc.). The viability of bacilli in the individual MΦ monolayers and in the MΦ detached from the monolayers are shown separately and as a total. These data show that only a few *M. leprae* were released or infected cells detached into the supernatant media, and the cumulative radio-respirometry (RR) results from individual wells confirmed the ability of IL-10 treatment to sustain intracellular viability of *M. leprae*.
Morphological evaluation of *M. leprae* with sustained metabolic activity in MΦ.

The morphological characteristics of *M. leprae* maintained in prolonged culture in mouse peritoneal MΦ were observed with light and electron microscopy. Elongated *M. leprae* were only observed under conditions where infected MΦ were maintained at 31°C in the presence of IL-10. As shown in Fig. 4, acid fast staining of infected MΦ at 4 weeks revealed that at 31°C in the presence of IL-10, many of the intracellular *M. leprae* were clearly elongated in comparison to those seen at 0 time or in MΦ maintained at 31°C without IL-10 (Figure 4A, B, C). At 37°C elongation of bacilli was not observed regardless of the presence of IL-10 (Fig. 4D). Under the transmission electron microscope (TEM), elongation was even more apparent. Not all bacilli in the 31°C, IL-10 group were observed to be elongated, as this required all bacilli to be sectioned through their long axis; but examination of dozens of infected cells in 2 experiments revealed elongated cells (8 to 10 µ) only in the 31°C, IL-10 group. *M. leprae*...
in the control group were consistently 2 to 4 µm in length (Fig. 5).

**DISCUSSION**

Our goals in this study were limited. Convincing evidence of actual intracellular multiplication of *M. leprae* would require at least a 10-fold increase in bacillary numbers. With a calculated multiplication cycle of 12.8 days in the mouse footpad model (37), this minimally acceptable increase in numbers would be difficult to demonstrate in a few weeks of MΦ tissue culture. However, the present study did show that the metabolism, and presumably the viability (42), of *M. leprae* could be sustained under culture conditions which also appeared to support the intracellular elongation of the leprosy bacillus.

*In vivo* *M. leprae* is able to enter and survive in a wide variety of tissues and cell types (24). Attempts to culture *M. leprae* in tissue culture have included the use of numerous cell lines derived from humans, rats, and mouse tissue with no evidence of multiplication (10, 27). The MΦ, the preferred host cell for the leprosy bacillus, offers an advantage over tissue culture cell lines since MΦ actively phagocytize *M. leprae* and, unlike cell lines, MΦ in culture are non-dividing adherent cells. Consequently the intracellular status of *M. leprae* over time is not confounded by an increase in host cell numbers. Chang and Neikirk (5) demonstrated the long term infection of mouse MΦ cultures with *M. leprae*, and a report of success in culturing *M. leprae* in MΦ was made by Garbutt (16), but was not confirmed by McRae and Shepard (27). Others reported limited, questionable, and unconfirmed success at detecting multiplication of the organism in MΦ cultures (8, 31, 34, 41). An exhaustive but unsuccessful attempt to cultivate *M. leprae* in tissue culture was made by Sharp and Banerjee (16) who employed MΦ from conventional mice and rats, nu/nu mice and rats and armadillos, rather than dividing cells and cell lines. Their *M. leprae* inocula was derived from 3 sources (human leproma, nu/nu mouse footpad and frozen infected armadillo tissue). Incubation temperature was varied from 31°C to 35°C and infected cells were maintained for up to 200 days. They rigorously evaluated any increase in leprosy bacilli and concluded that no significant multiplication occurred.

Our studies provide groundwork for fu-
ture rational attempts to cultivate \textit{M. leprae} with notable advantages to our approach. Most importantly, our starting inoculum of \textit{M. leprae} was freshly obtained for each experiment from infected nu/nu mice maintained under conditions designed to maximize \textit{M. leprae} viability \cite{12}. We also were able to rapidly quantify the metabolic activity of \textit{M. leprae} using the RR technique adapted by Franzblau \cite{13}. This assay can readily detect activity from as few as 10^6 bacilli with the results available in 1 wk (compared to 6 to 12 months when titrated in mouse footpads). RR data correlates well with other \textit{in vitro} systems \cite{13} but, more importantly, as shown in a recent series of 36 separate experiments, RR metabolic data correlated well with “viability” studied in the so-called “gold standard” mouse footpad system \cite{12}.

Whether intracellular or extracellular, \textit{M. leprae} clearly prefers temperatures cooler than normal human body temperature, lending experimental credence to the clinical observations of generations of leprologists regarding the distribution of \textit{M. leprae} in the cooler, permissive sites in human disease, the skin and mucous membranes of the upper respiratory tract. These studies also confirm and extend the results of dozens of other \textit{in vitro} experiments \cite{42} where 37°C appeared to be highly detrimental to \textit{M. leprae} viability.

Under conditions of an effective cellular immune (CMI) response, MΦ are likely a major anti-microbial effector cell in host capacity to cope with the leprosy bacillus. We have previously shown that mouse MΦ, activated by interferon (IFN-\(\gamma\)), kill or inhibit a wide variety of intracellular pathogens \cite{36}, including \textit{M. leprae} \cite{32}. Enhanced fusion of secondary lysosomes with \textit{M. leprae}-containing phagosomes occurs in activated MΦ \cite{19} and is accompanied by the enhanced production of reactive oxygen (ROI) and nitrogen intermediates (RNI) as potent anti-microbial mechanisms \cite{4}. We have subsequently shown \textit{in vivo} in transgenic knock-out mice and \textit{in vitro} in MΦ from these mice that ROI are relatively ineffective in comparison to the potent effects of RNI in host defense against \textit{M. leprae} \cite{1,4}. However, although the enhanced production of RNI by activated MΦ is likely a principal antimicrobial mechanism, in each of our studies, normal MΦ produced a measurable baseline level of RNI, insufficient to rapidly kill bacilli but perhaps sufficient to inhibit the long term viability of the fastidious, intracellular leprosy bacillus.

In choosing TGF and IL-10 as the cytokines that might bolster the intracellular survival of \textit{M. leprae}, we were attempting to down regulate any innate ability of the normal MΦ to cope with the organism. TGF-\(\beta\) is produced by activated MΦ and other inflammatory cells and has a broad array of modulatory functions on the immune response. TGF-\(\beta\) has been shown to interfere with MΦ antimicrobial mechanisms including generation of ROI \cite{43} and RNI \cite{7}, and has been shown to enhance the intracellular growth of \textit{M. tuberculosis} in human monocytes \cite{23}. However, as employed in the present studies with mouse MΦ exogenous TGF-\(\beta\) had no detectable effect on sustaining intracellular \textit{M. leprae} viability, a finding perhaps attributable to the enhanced innate antimicrobial ability of human monocytes in comparison to human monocyte derived MΦ \cite{44} which are more akin to resident mouse peritoneal MΦ.

In contrast, supplementing media with IL-10 clearly affected the long term viability of \textit{M. leprae} in mouse MΦ. IL-10 is produced in TH1 responses by T cells, B cells and MΦ \cite{11,28}. IL-10 has been shown to be a potent down-regulator of CMI to intracellular pathogens \cite{33}. \textit{In vivo}, endogenous IL-10 dampened the CMI response to avirulent mycobacterial infection \cite{35} and appeared to lead to loss of control of \textit{M. tuberculosis} infection with widespread dissemination \cite{9}. IL-10 functions in part at the level of the macrophage by attenuating inducible nitric oxide synthase (iNOS) mRNA expression, iNOS activity and, by inference, NO production \cite{22}. \textit{In vitro}, exogenous IL-10 inhibited production of nitric oxide (NO) in MΦ infected with \textit{Babesia} merozoites \cite{17}. In our own studies, IL-10 did markedly inhibit the production of NO by IFN-activated MΦs but any inhibition by IL-10 of baseline production of NO by normal MΦs was below the limits of detection of the NO\textsubscript{assay} (data not shown). Exogenous IL-10 also interferes with IFN-induced antimycobacterial MΦ activities as shown in studies with \textit{M. bovis} \cite{12}.

In addition to sustained metabolism of \textit{M.}}
leprae, we also explored a morphological parameter of M. leprae vitality within cultured macrophages, bacillary elongation. Nakamura, et al. reported the elongation of M. leprae-murium in culture medium (28), and in 1969 Chang and Anderson (4) evaluated intracellular growth of M. leprae-murium in cultured mouse peritoneal ΜΦ over a 12 to 17 week period and observed marked elongation of bacilli well before the appearance of bacillary multiplication. In our studies, in addition to sustained or enhanced metabolism, intracellular elongation of individual bacilli was observed after 4 weeks culture in murine ΜΦ maintained with IL-10. Elongation was first observed with the light microscope and subsequently confirmed with the transmission electron microscope. A drawback to the use of the TEM for such observations is that unlike light microscopy which revealed the bacilli at their full intracellular length, processing for the TEM required sectioning infected ΜΦ with no certainty that the full length of each bacillus was cut. Nevertheless sufficient numbers of clearly elongated bacilli were seen to confirm the light microscopy findings. Similarly, in preliminary studies with armadillo peripheral blood monocyte derived ΜΦ maintained at 33°C for 4 weeks, intracellular M. leprae were predominantly elongated (data not shown).

Septal formation has been described in TEM studies from human leprosy biopsies by Hirata (20) and was occasionally observed by us in individual bacilli under the TEM after 4 weeks of incubation of infected mouse ΜΦ in the presence of IL-10. The small sample size precluded quantification but septa appeared to be observed far less frequently in bacilli in the control ΜΦ. Septal formation in M. leprae murium in the mouse model has been reported to indicate dividing stage of the bacillus, and Hart and Rees (19) concluded that elongation in vitro was an inherent feature of M. leprae murium that distinguishes it from M. leprae although it is likely that the M. leprae inocula employed were of very low viability. Experiments are currently underway to study M. leprae in our cell culture system employing an environmental scanning electron microscope which by passes the need for critical point drying and its attendant artifacts and will permit quantitation of elongation.

Further work with infected armadillo ΜΦ is also clearly warranted. Other than having a core temperature of ~33°C, little is known about the unique characteristics of Dasypus novemcinctus, the nine-banded armadillo, that render it as a permissive host for the leprosy bacillus (25). In vivo, mononuclear phagocytes in virtually every organ of the natural or experimentally infected armadillo become heavily parasitized with propagating M. leprae (26).

The inability to culture M. leprae has undoubtedly hindered almost every aspect of leprosy research, and thus, our understanding of this disease lags behind that of many others of bacterial etiology. These promising results represent only preliminary findings, but suggest that this approach of inhibiting the innate anti-microbial properties of the ΜΦ to bolster the intracellular survival of M. leprae may ultimately provide clues allowing the long sought-after cultivation of the leprosy bacillus. In vitro cultivation of M. leprae could make available for the first time, large quantities of pure bacilli produced inexpensively under defined conditions. Thus, large amounts of purified antigens would be available for basic and applied immunological studies, including the development of specific skin test antigens and vaccine preparations. The time and cost of screening new drugs and susceptibility testing of clinical isolates would also be greatly reduced. Our understanding of leprosy epidemiology might increase by determining the existence of human carriers, non-human reservoirs, or environmental sources of M. leprae. Phenotypic variation among cultured worldwide isolates could become feasible as might the generation and characterization of mutants. Finally, cultivation of M. leprae, in concert with the genome project, would clearly enhance our understanding of the physiology of this fastidious pathogen, including elucidation of metabolic pathways, studies of virulence mechanisms, drug resistance and the factors underlying “persistence.”

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Detection of IL-13, IL-10, and IL-6 in the Leprosy Skin Lesions of Patients during Prednisolone Treatment for Type 1 (T1R) Reactions

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ABSTRACT

This study demonstrates the presence of IL-10 and IL-6, by immunohistochemistry, in the skin lesions of patients with Type 1 reactions. Fifteen patients with Type 1 reaction from Hyderabad, India were included in this study. They were all receiving standardized treatment for Type 1 reactions: a reducing course of daily oral prednisolone for 6 months. Biopsies were taken before treatment and during treatment at weeks 1, 4, and 6 months. IL-13 was observed in the lesions of most patients. By week 4 of treatment, the presence of IL-13, IL-10, and IL-6 in the lesions had decreased significantly.

Although some patients showed significant clinical skin sign improvement within one week of therapy, no concomitant decrease or increase in any of the cytokines was observed at this time point. Interestingly, some cytokine activity within the lesions was observed after 6 months of treatment.

RÉSUMÉ

Cette étude met en évidence par immuno-histochimie la présence d’IL-10 et d’IL-6 au sein de lésions de patients souffrant de réaction de type 1. Quinze patients présentant des réactions de type 1, provenant de Hyderabad aux Indes, ont été recrutés pour cette étude. Ils étaient tous en train de recevoir un traitement standard de la réaction de type 1 : une dose décroissante de prednisolone par voie orale pendant 6 mois. Les biopsies ont été effectuées avant le traitement et aux semaines 1, 4 et 6 mois de traitement. L’IL-13 fut observée dans les lésions de la plupart des patients. Vers 4 semaines de traitement, la présence de l’IL-13, IL-10 et l’IL-6 avaient diminué de façon significative.

Bien que certains patients aient montré une amélioration significative des signes cliniques dans la semaine qui a suivi le début du traitement, il n’y avait pas à ce temps de prélèvement d’évidence suggérant une augmentation ou une diminution d’une de ces cytokines. De façon surprenante, certaines activités de cytokines furent observées après 6 mois de traitement au sein des lésions.

RESUMEN

Se hizo un estudio inmunohistoquímico en las lesiones de la piel de pacientes con reacción leprosa Tipo 1 para buscar la presencia intraleional de IL-13, IL-10 e IL-6. Se incluyeron 15 pacientes de Hyderabad, India, con lepra y reacción leprosa Tipo 1. Todos los pacientes estaban recibiendo el tratamiento estándar para este tipo de reacción: dosis diarias decrecientes de prednisona durante 6 meses. Se tomaron biopsias antes y durante el tratamiento a las semanas 1, 4 y 24 (6 meses). Al inicio del tratamiento, las lesiones de la mayoría de los pacientes mostraron la presencia de IL-13, sin embargo, cuatro semanas después, la expresión de IL-13, IL-10 e IL-6 en las lesiones había disminuido significativamente.
Type 1 reactions (T1R) occur in about 30% of patients with the immunologically unstable borderline forms of leprosy. These reactions are phases of acute inflammation that often lead to nerve damage and are associated with high levels of pro-inflammatory cytokines in the skin and nerve (6). Six months of treatment using the corticosteroid, prednisolone, reduces skin inflammation and improves nerve function in about 60% of patients (9). Although T1R episodes are described as phases of delayed-type hypersensitivity (DTH) associated with the clearance of mycobacteria (7), the immunological factors underlying these reactions and specifically nerve damage are not well understood. Research in this field is required to understand the complex immunology underlying T1R so that treatment for these patients may be improved.

Serum levels of both pro- and anti-inflammatory cytokines decrease during multidrug therapy (MDT) but this is reversed during the onset of a T1R (13). In the skin lesions of these patients, T1R episodes are associated with the expression of many cytokines including many inflammatory: interleukin-12 (IL-12), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and anti-inflammatory cytokines: interleukin-10 (IL-10), T-cell growth factor-β (TGF-β) (6, 11, 12). The “upgrading” of the protective cell mediated immunity in T1R is paradoxically associated with the neuropathy observed in these patients. In particular, the expression of TNF-α as part of the antimicrobial response is considered to be a key mediator associated with the systemic inflammatory symptoms observed in T1R (6).

The role that prednisolone plays in controlling nerve damage is partially due to its capacity to down-regulate the pro-inflammatory cytokines. The effect of prednisolone on pro-inflammatory cytokines has been observed in many severe inflammatory diseases (3, 5). The down-regulatory effect of prednisolone on inducible nitric oxide synthase (iNOS), IFN-γ, IL-12, TNF-α, and IL-6 in the lesions as been demonstrated (8, 12). However, the effect of prednisolone on other regulatory cytokines is not clear, although there is some in vitro evidence that high doses of methylprednisolone increase LPS-induced IL-10 levels (10). In leprosy reactions, no reduction in the expression of IL-10 has been observed during treatment (14). With the effects of prednisolone on inflammatory cytokines well documented, this study aims to determine whether prednisolone effects the expression of the regulatory cytokines IL-10, IL-13, and IL-6 in the skin, and determines whether there is a direct relationship between the expression of these and the clinical skin sign improvement in patients with T1R.

MATERIALS AND METHODS

Patients. Fifteen leprosy patients with T1R (borderline tuberculoid: n = 6, borderline lepromatous: n = 9) from Blue Peter Research Center (BPRC), Hyderabad, India were recruited for this study. Patient status according to Ridley-Jopling (15) scale and MDT treatment is given in The Table and in a previous publication (6). T1R was defined as the appearance of erythema and oedema in either existing or new leprosy skin lesions within the previous 2 weeks. Histological examination of biopsies was undertaken for all patients. Patient consent was obtained before collection of biopsies. Skin biopsies (6 mm) were taken from the reactive skin lesions at time points during treatment, and snap frozen and stored in liquid nitrogen. The initial biopsy was taken at the time of presentation before prednisolone treatment had commenced (day 0). Subsequent biopsies were taken from the lesion (close to the previous biopsy site) after weeks 1, 4, and 26. Treatment consisted of a standard reducing course of steroids, initially of 30 mg oral prednisolone daily, which was reduced by 5 mg each month for 6 months. Clinical improvement in this study relates to improvement seen in the skin signs of the patients. Skin signs were measured by applying a numerical severity scale that assessed the degree of inflamma-
PLEX (ABC) methods (Fig. 1). Sections were incubated with the following antibodies: monoclonal anti-IL-13 (Peprotech, St. James Square, London, U.K.); monoclonal anti-IL-10 (Santa Cruz Biotechnology Ltd., California, U.S.A.); monoclonal anti-IL-6 (Santa Cruz Biotechnology Ltd., California, U.S.A.). An irrelevant murine antibody of the same isotype was included as a negative control. Positive staining was developed using 3,3′-diaminobenzidine (DAB) chromogen (Sigma) and counterstained in haematoxylin (Sigma). Cellular infiltration and cytokine staining were measured on scales described in The Table legend and previously (8). All cytokine staining was evaluated independently by two observers. Differences between all four groups were determined statistically using the Student’s t test.

RESULTS

Cellular infiltration. Cryosections (thickness 5 to 6 µm) were adhered to silane (Sigma, Poole, U.K.)-coated slides and acetone or paraformaldehyde (4%, Sigma) fixed. Non-specific protein binding was prevented by incubation in serum-free blocking medium (Dako, Glostrup, Denmark). Staining was refined using the peroxidase-antiperoxidase (PAP) and avidin-biotin complex (ABC) methods (Fig. 1). Sections were incubated with the following antibodies: monoclonal anti-IL-13 (Peprotech, St. James Square, London, U.K.); monoclonal anti-IL-10 (Santa Cruz Biotechnology Ltd., California, U.S.A.); monoclonal anti-IL-6 (Santa Cruz Biotechnology Ltd., California, U.S.A.). An irrelevant murine antibody of the same isotype was included as a negative control. Positive staining was developed using 3,3′-diaminobenzidine (DAB) chromogen (Sigma) and counterstained in haematoxylin (Sigma). Cellular infiltration and cytokine staining were measured on scales described in The Table legend and previously (8). All cytokine staining was evaluated independently by two observers. Differences between all four groups were determined statistically using the Student’s t test.

THE TABLE  BL-RR, borderline lepromatous leprosy in reversal reaction; BT-RR, borderline tuberculoid leprosy reversal reaction.*

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<th>Sex</th>
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*MDT therapy, multi-drug therapy; PB, paucibacillary bacillary regimen; MB, multibacillary regimen; (1) completed MDT before reaction; (2) on MDT at time of reaction; (3) no previous history MDT and given MDT during study. —, not known. Scoring for skin signs described in text. Scoring for cytokine staining was as follows: 0, negative; 1, <10% of cells staining positive; 2, 10 to 30% of cells staining positive; 3, 50 to 80% of cells staining positive; and 4, 80 to 100% of cells staining positive. Scoring for cellular infiltrate was as follows: 0, no cellular infiltrate; 1, small granulomas; 2, medium-sized granulomas; and 3, large granulomas.

b Samples collected on day 0; and after 1 week, 4 weeks, and 26 weeks of treatment. The 26 week time point varied between 25 and 27 weeks and some patients were unavailable for sampling.
cellularity and size of these granulomas decreased with treatment (Fig. 2). This decrease was significant between day 0 and week 4 (p <0.001) and between week 1 and week 4 (p <0.005) of treatment. No significant difference in cellularity was found within the first week of treatment. No significant difference was found when comparing the borderline lepromatous (BL) and borderline tuberculoid (BT) groups (results not shown).

Cytokine expression. At the first time point all patient biopsies had strong positive staining for IL-6 and IL-10. IL-13 expression was observed in 11 out of 15 patients at the first time point (The Table). Analysis of all the biopsies revealed that the mean expression of IL-6, IL-10, and IL-13 decreased, but only significantly after 4 weeks of treatment (Fig. 3). The mean level of expression of IL-6 decreased significantly between day 0 and weeks 4 (p <0.005) and also between week 1 and week 4 (p <0.002) (Fig. 3). Similarly, the level of expression of IL-10 decreased between day 0 and week 4 (p <0.001), and between week 1 and 4 (p <0.0001) but no alteration in IL-10 levels was observed after one week of treatment. The percentage of cells that stained positive for IL-13 was lower than that of IL-6 or IL-10 (Fig. 3). In accord with the IL-10 and IL-6 results, the only significant drop in IL-13 cytokine expression was observed between week 1 and week 4 (p <0.05) and between day 0 and week 4 (p <0.005) but not in the first week of treatment or between week 4 and week 26. Two patients (179 and 141) had a high level of expression of IL-10 and IL-6 over the full 6 months of treatment. Taking each time point there was no significant difference between the BT and BL patients for any of the cytokines (results not shown).

Clinical improvement. The clinical improvement of the patients was evaluated by the skin sign grading (The Table). When analyzing all patients, significant skin sign improvement was observed after 4 weeks of treatment and between week 1 week and week 4 (p <0.002). Clinical improvement after 4 weeks of prednisolone treatment corresponds to decreased cellularity and the reduced expression of IL-6, IL-13, and IL-10 observed in the skin biopsies. Nine out of the 15 patients showed significant clinical improvement within the first week of treatment. In the lesions of these patients, no associated difference was observed in the in vivo expression of any of the IL-6, IL-10, or IL-13 (Fig. 4a). Six of the 15 patients showed no skin sign improvement in the first week of treatment. These patients showed no associated differential in the observed expression of IL-6, IL-10, or IL-13 (Fig. 4b).

DISCUSSION

Type 1 reactions (T1R) are acute inflammatory events usually occurring in borderline leprosy patients with the immunologically unstable form of leprosy. The expression of inflammatory cytokines, in particular TNF-α, is thought to be responsible for the pathological nerve damage associated with T1R (6,8,17). To study the role of other cytokines within the skin lesion granulomas of T1R patients, levels of IL-10, IL-13, and IL-6 have been evaluated by immunohistochemistry.

Although previous studies have provided evidence of anti-inflammatory cytokines at
Atkinson et al.: Detection of IL-13, IL-10, and IL-6 for type 1 reaction

Atkinson et al.: Detection of IL-13, IL-10, and IL-6 for type 1 reaction

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Atkinson et al.: Detection of IL-13, IL-10, and IL-6 for type 1 reaction

Atkinson et al.: Detection of IL-13, IL-10, and IL-6 for type 1 reaction

Atkinson et al.: Detection of IL-13, IL-10, and IL-6 for type 1 reaction

Atkinson et al.: Detection of IL-13, IL-10, and IL-6 for type 1 reaction

the transcriptional level (12), this study confirms the presence of IL-10 and IL-13 protein in the granulomas of the skin lesions of patients with T1R. The presence of IL-6 in these lesions has also been confirmed. The presence of both anti- and pro-inflammatory cytokines in these lesions highlights the multiplicity of cytokine expression within the granulomas of these patients and suggests that a simple model of Th1 activation is insufficient to explain reactional pathology. These results also highlight the true complexity of regulatory pathways within the granuloma. Other inflammatory diseases are also characterised by the expression of both pro- and anti-inflammatory cytokines (16).

Pro-inflammatory cytokines have a num-

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**Fig. 2.** Representative photomicrographs of tissue sections taken from lesions of a T1R patient (no. 260). Sections were stained with immunoperoxidase for IL-6. Strong positive staining was seen at week 0 Fig. 2a (top-left) and week 1 Fig. 2b (top-right) with less positive staining in weeks 4 Fig. 2c (bottom-left) and 26 Fig. 2d (bottom-right). 40× objective.
number of effects within the granuloma (i) promoting cellular protective responses, (ii) maintaining granuloma formation \(^2\), and (iii) initiating nerve damage \(^6\). In contrast, the role of anti-inflammatory cytokines within the granuloma is not well understood. It can be postulated that the anti-inflammatory cytokines play a crucial role in controlling the critical balance between the protective and tissue damaging effects of the pro-inflammatory cytokines. Within the granuloma the control of the network of cytokines appears to be self-regulating. The expression of these anti-inflammatory cytokines within the lesions of patients with T1R highlights the high degree of cellular activity and cytokine expression occurring within the granuloma.

Current treatment for T1R is designed to inhibit the production of pro-inflammatory mediators. Prednisolone is an effective inhibitor of pro-inflammatory cytokines in leprosy reactions and other inflammatory diseases. The effect of prednisolone on anti-inflammatory mediators is not so well understood although it has been demonstrated that prednisolone promotes anti-inflammatory cytokines in asthma patients \(^4\). This study provides evidence that prednisolone down regulates the expression of IL-10, IL-6, and IL-13 within the lesions of patients with T1R. This down regulation could be due to the decrease in cellular activity during pro-inflammatory down regulation within the granuloma, or via the direct effect of prednisolone on transcription factors such as NF-kB \(^1\). The development of an effective treatment should focus on directing the control and balance of these cytokines.

To detect a correlation between clinical presentation and cytokine expression, the skin sign improvement scale was analyzed against the level of the cytokine expression. Within the first week of treatment, improvement in the clinical skin sign was observed in nine of the fifteen patients. Surprisingly, an associated change in the production of any of the cytokines within the skin lesion granulomas of these patients was not observed until week 4 of treatment. Similarly, deterioration in the clinical skin signs within the first week is not associated with any of the cytokines measured. This suggests that skin sign clinical improvement is not directly mediated by the expression of these cytokines within the granulomas of the skin lesions but that skin sign clinical improvement is mediated by events other than those related to cytokine expression. The skin sign improvement may be associated with other known effects of prednisolone, such as the effect on prostaglandin levels \(^4\). Previous work \(^8\) has shown that IL-12, IFN-\(\gamma\), and iNOS protein within the lesions of the same patients reduced in expression in a similar manner as
the cytokines measured in this study. The fact that the expression of all of these cytokines within the granuloma is not seen until 4 weeks after the start of treatment may be due to both the complexity of the cellular organisation and the cytokine pathways network within the granuloma.

Although the level of cytokine activity within the granulomas declines within 4 weeks of therapy, this activity is not completely abrogated by 6 months. IL-12, IFN-γ, and iNOS were also observed in the lesions of some these patients after 6 months of treatment (9). This suggests that cytokine activity within the granuloma may be difficult to switch off and/or the granuloma may be semi-pervious to prednisolone or that the circulating levels of prednisolone are not high enough to affect granuloma activity.

These findings have implications for the treatment of reactional patients. If the pro-inflammatory cytokines are involved in the peripheral neuropathy, the need for a fast acting anti-inflammatory treatment is required. Although the up-regulation of pro-inflammatory cytokines is directly involved in reactional lesions, this study provides evidence that the anti-inflammatory cytokines may also play a role in the immunopathology of T1R. In particular, further studies should focus on the use of inhibitors controlling the overall balance of pro- and anti-inflammatory cytokines rather than on specific cytokine inhibitors.

It should be noted that twelve of the patients were receiving MDT which includes the mild anti-inflammatory drug clofazamine. The effect of this drug can not be determined within this sample size.

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REFERENCES


Leprosy Patients with Lepromatous Disease Have an Up-regulated IL-8 Response That Is Unlinked to TNF-α Responses

Z. Hasan, A. Mahmood, S. Zafar, A. A. Khan, and R. Hussain

ABSTRACT

Tumor necrosis factor (TNF-α) in conjunction with interferon-γ (IFN-γ) plays an important role in lymphocyte recruitment and granuloma formation in mycobacterial diseases. Lepromatous leprosy infections are typically associated with low to absent T cell responses and the absence of INF-γ secretion. Chemokines such as IL-8, MCP-1, and MIP-1β, have also been shown to recruit neutrophils and lymphocytes to the site of mycobacterial infections. We have studied IL-8 expression in relation to TNF-α and TGF-β in monocytes from lepromatous patients (LL) as compared with healthy endemic controls. In endemic controls, no spontaneous expression of IL-8, TNF-α, and TGF-β was observed, but BCG and M. leprae induced activation of all three cytokines. Lepromatous leprosy monocytes spontaneously expressed high levels of IL-8 and TGF-β but negligible levels of TNF-α. A further increase in IL-8 secretion or gene expression by BCG or M. leprae was not significant. BCG, but not M. leprae, was able to stimulate TNF-α activation in lepromatous leprosy subjects. TGF-β responses in LL were parallel to those of IL-8. This suggests a vigorous and active ongoing IL-8 response in lepromatous disease that is independent of TNF-α activation. Therefore, in the absence of IFN-γ and TNF-α activation, IL-8 may assume a pivotal role in cell recruitment in leprosy patients with disseminated mycobacterial infections.

RÉSUMÉ

Le facteur de nécrose tumoral (TNF-α), associé à l’interféron-γ (IFN-γ), joue un rôle important pour le recrutement des lymphocytes et la formation des granulomes dans les maladies à mycobactéries. Les infections de lèpre lépromateuse sont habituellement associées à des réponses faibles à nulls de lymphocytes T et une absence de sécrétion d’IFN-γ. Il a aussi été démontré que les chimiokines comme l’Il-8, la MCP-1 et le MIP-1β pouvaient permettre le recrutement de neutrophiles et de lymphocytes au sein des sites d’infection par les mycobactéries. Nous avons étudié ici l’expression de l’IL-8, en relation avec TNF-α et TGF-β, des monocytes de patients lépromateux (LL) et de témoins sains en zone endémique. Chez les témoins de zone endémique, il ne fut pas observé d’expression spontanée d’IL-8, de TNF-α ni de TNF-β, cependant tant le BCG que M. leprae ont provoqué l’activation de ces 3 cytokines. Les monocytes des patients souffrant de lèpre lépromateuse exprimaient spontanément de hauts niveaux d’IL-8 et de TGF-β mais une quantité négligeable de TNF-α. L’augmentation de sécrétion ainsi que la sur-expression du gène de l’IL-8 ne s’est pas avérée significative, après stimulation par le BCG ou M. leprae. Le BCG, mais pas M. leprae, a été capable de stimuler l’activation de TNF-α parmi les sujets atteints de lèpre lépromateuse. Les réponses de TGF-β chez les LL étaient très similaires à celles de l’IL-8. Cela suggère qu’il existe une médiation vigoureuse, active et continue de l’IL-8 dans la maladie lépromateuse, qui est indépendante de l’activation du TNF-α. L’IL-8 pourrait donc bien assurer un rôle central de recrutement cellulaire chez les patients atteints de lèpre présentant une infection massive et disséminée, en l’absence d’activation de l’IFN-γ et de TNF-α.

RESUMEN

El factor de necrosis tumoral alfa (TNF-α), junto con el interferón gamma (IFN-γ), juega un papel importante en el reclutamiento de linfocitos y en la formación de granulomas en las
Leprosy is a spectral disease with severity ranging from the localized or tuberculoid form to the disseminated or lepromatous form at the two poles, with several intermediate forms. The presentation of these forms is determined by the varying immune status of the infected individual (27). At the tuberculoid pole the disease is more restricted and bacilli tend to be contained in well-defined granulomas. At the lepromatous pole the disease is characterized by diffuse multibacillary lesions containing an extensive bacterial load in tissue macrophages especially, in the skin and also in Schwann cells of the peripheral nerves. Cytokine activation is critical to recruitment of a protective inflammatory response by macrophages and other polymorphonuclear leukocytes (PMNs) at the site of infection in the initial stages of the disease, for granuloma formation and limiting spread of infection. Monocytes-derived tumor necrosis factor alpha (TNF-α) (16) and T-cell derived interferon gamma (IFN-γ) play a pivotal role in the restriction of Mycobacterium leprae infections (13). In chronic disseminated lepromatous disease there is T cell anergy and low to absent IFN-γ responses as well as low levels of TNF-α secretion from peripheral blood mononuclear cells (PBMCs) in response to either mitogenic (7) or M. leprae components (7), as compared with the tuberculoid form of the disease.

In lepromatous patients, chemokines may assume a greater role in cell recruitment where there are low levels of T cell and monocyte derived pro-inflammatory cytokines. Leucocyte-attracting chemokines such as, the CXC chemokine interleukin-8 (IL-8), macrophage chemotactic protein-1 (MCP-1), and macrophage inhibitory protein-1β (MIP-1β), produced by macrophages, epithelial and mast cells have been shown to play a role in recruitment of neutrophils and lymphocytes in mycobacterial infections (14, 19, 32). IL-8 is stimulated in response to Mycobacterium tuberculosis and its cellular components (23, 31, 39, 41), M. bovis (24) and M. avium (88). It has been shown to selectively recruit effector leucocyte populations to tuberculose granulomas (9). However, little is known about the role of chemokines in disseminated leprosy. In the current study we have focused on IL-8 responses in relation to TNF-α in both uninfected and infected (BCG and M. leprae) monocytes from lepromatous patients and compared them with healthy controls. In addition, we determined expression of the pleiotropic cytokine TGF-β which is thought to be immunosuppressive role in leprosy (15) and tuberculosis (12) infections. We found selective up-regulation of IL-8 but not of TNF-α in patients with lepromatous leprosy disease (LL), concomitant with increased TGF-β. Infection with BCG or M. leprae activated IL-8 responses but this was not significant due to the high basal level of IL-8. Mycobacterium-induced TNF-α activation was reduced as compared with endemic controls. These results suggest that IL-8 responses are active in these anergic patients and may contribute to disease pathology.

MATERIALS AND METHODS

Leprosy subjects and controls. Leprosy patients were recruited in collaboration
with the Marie Adelaide Leprosy Center, Karachi, Pakistan, and classified based on clinical presentation using World Health Organization (WHO) criteria (30). Fourteen subjects with lepromatous leprosy (LL), thirteen males and one female, were included in the study with informed consent. The age range of patients was 25 to 65 yrs with a mean of 34.4 yrs. Bacterial index (BI) of patients was assessed by slit skin smears. Patients were further divided into a short-term (ST) treatment group of MDT ≤1 month or, a long-term (LT) treatment group of multi-drug therapy (MDT) 2 to 6 months. Their clinical characteristics are further described in Table 1. BCG-vaccinated healthy volunteers (N = 14), 10 males and 4 females, ranging 25 to 50 yrs, with a mean age 32.4 yrs were included as a control group.

**Mycobacterial strains.** *M. bovis* BCG (Montreal vaccine strain) was kindly provided by Dr. Douglas Young, Imperial College, U.K. Mycobacteria were grown to logarithmic phase in 7H9 Middlebrook medium supplemented with 0.02% glycerol, 10% ADC Middlebrook enrichment and 0.5% Tween-80 (Difco Laboratories, Detroit, MI, U.S.A.). BCG were quantitated by growth on 7H10 Middlebrook agar, supplemented with 0.02% glycerol and 10% OADC Middlebrook enrichment (Difco Laboratories, Detroit, MI, U.S.A.). Mycobacteria were frozen in growth medium containing 20% glycerol as single use aliquots at –70°C. On recovery, the frozen stocks were found to be greater than 95% viable as determined by colony forming units (CFUs) and flouresceindiacetate-ethidium bromide staining (18). For the infection assays, aliquots of BCG were freshly thawed, washed 3 times in phosphate buffered saline (PBS) and diluted as required for the infection. To avoid clumping of mycobacteria, the cell suspension was sonicated briefly then allowed to stand for 5 min to allow large clumps to settle, leaving behind a mainly single cell suspension (as determined by acid-fast staining). Gamma-irradiated *M. leprae* (prepared from armadillo liver tissue) was provided by Dr. Patrick Brennan, Colorado State University, U.S.A., by National Institute of Health (NIH) contract (NO1-AI-55262, “Leprosy research support”) as a 1 mg/ml stock containing approximately 2.9 × 10⁹ mycobacteria/ml. The *M. leprae* was stored in single use aliquots at –70°C, and was washed in PBS, diluted as required as described above for the infection assays.

**Isolation and infection of monocytes.** PBMCs were obtained by gradient separation of whole blood using Histopaque (Gibco BRL, NY, U.S.A.). Monocytes were isolated by adherence for 1.5 hr at 37°C after which cells were harvested using Cell Dissociation Solution (Sigma), counted and seeded at 5 × 10⁵ per well in 24-well plates. Monocytes were cultured for 18 to 20 hours in RPMI 1640 medium containing heat-inactivated 10% human AB Serum (Sigma) and 2.5 mM glutamine. An optimal mycobacterium:monocyte ratio was first determined for the infection assays as described previously (11). Subsequent infection assays were carried out at a ratio of 10 bacteria per cell.

**ELISA for TNF-α and IL-8.** TNF-α standards and monoclonal antibodypairs for capture and detection were obtained from Pharmingen (San Diego, CA, U.S.A.). TNF-α was measured using a sandwich ELISA technique according to the manufacturer’s recommendation and as reported previously (13). IL-8 detection reagents were obtained from R&D Systems, U.S.A., and were also used in a sandwich ELISA method according to manufacturer’s recommendation. The range of detection was between 7.8 to 1000 pg/ml. Each sample was tested in duplicate. Results are expressed as mean values from at least four independent experiments after deduction of spontaneous cytokine production. As expected, donor to donor variation was present but results were consistent and were pooled for data analyses.

### Table 1. Characteristics of lepromatous leprosy (LL) patients included in study.

<table>
<thead>
<tr>
<th></th>
<th>No. of subjects (N)</th>
<th>Average age (yrs)</th>
<th>Mean BI</th>
<th>Mean MDT (mos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term treatment group (LL-ST)</td>
<td>7</td>
<td>29.9</td>
<td>2.75</td>
<td>0.28</td>
</tr>
<tr>
<td>Long-term treatment group (LL-LT)</td>
<td>7</td>
<td>35</td>
<td>3.18</td>
<td>3.92</td>
</tr>
</tbody>
</table>

BI, bacterial index at time of enrollment in the study; MDT, duration of multi-drug treatment at time of enrollment.
**Statistical analyses** were carried out using the Microsoft EXCEL Program. Values in each group of results were compared using ANOVA analysis. Un-paired Student’s *t*-test was carried out with 95% confidence intervals so that *p*-values ≤0.05 were considered significantly different.

**RT-PCR for cytokines.** Cell monolayers were directly harvested in TriZOL reagent (GIBCO BRL) and total RNA, isolated according to the manufacturer’s recommendations. Reverse transcription of 1 to 2 µg RNA was carried out using oligo dT primers at 37°C for 2 hrs with Murine Leukemia Virus transcriptase (GIBCO-BRL). Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was carried out using sequence specific primers for the housekeeping β-actin gene, IL-8, TNF-α, and TGF-β. PCR was first carried out for β-actin and subsequently, equivalent cDNA template was used in the PCR reactions. Primers used were: β-actin-F 5′ GTG GGG CGC CCC AGG CAC CA 3′, -R 5′ CTC CTT AAT GTC ACG CAC GAT TTC 3′; TNF-αF (5′ TCT CGA ACC CCG AGT GAC AA 3′), -R (5′ TAT CTC TCA GCT CCA CGC CA 3′); TGF-β-F (5′ GCC CTG GAC ACC AAC TAT TGC 3′), -R (5′ GCT GCA CTT GCA GGC GCG CAC 3′) and commercially available primers for IL-8 (R&D systems, U.S.A.). PCR conditions for IL-8 and TNF-α were briefly: 94°C, 2 min; 30 cycles, 94°C, 45 seconds; 56°C, 45 seconds; 72°C, 1 min; 72°C, 10 min. Annealing temperatures for β-actin and TGF-β were 58°C. The mRNA products of sizes 540 bp (β-actin), 158 bp (IL-8), 124 bp (TNF-α) and 336 bp (TGF-β) were detected. Gene expression was normalized using β-actin, whereby the PCR products obtained were quantitated by scanning densitometry scanned using the BIORAD Gel Documentation system (BIORAD, U.S.A.), which provides arbitrary units for the relative intensity of each band obtained. Densitometry results were obtained for each test sample for cytokine PCRs and gene expression indicated as cytokine/β-actin ratios.

**RESULTS**

**Differential induction of IL-8 and TNF-α secretion in lepromatous patients.** We first determined basal levels of IL-8 and TNF-α expression in our group of lepromatous leprosy patients (LL) and in endemic controls. Very little spontaneous secretion of IL-8 was observed in healthy donors, while that in lepromatous patients was significantly elevated (*p* <0.05) (Fig. 1A). However, there was no spontaneous secretion of TNF-α observed in either healthy donors or patients (Fig. 1B). Since chemotherapy may change the immune profile, we also analyzed the effects of duration of treatment on cytokine secretion. There was no significant difference in IL-8 secretion in short term treated leprosy patients (LL-ST) or long-term treated leprosy patients (LL-ST), *N* = 6. The mean values are illustrated with S.E.M. as ‘y’ error. A. IL-8, B. TNF-α. Significance of differences between LL groups and ECs were determined using an unpaired Student’s *t*-test. *, *p* ≤0.05 as significant.
To determine whether cytokines was further inducible in monocytes from leprosy patients, we infected cells with live, attenuated *M. bovis* BCG or irradiated *M. leprae*. Previously, we have found 6 hr post-infection to be an optimal time to measure mycobacterium-induced TNF-α (11). For IL-8 we carried out a time course infection of monocytes at 1, 6, and 18 hr, and found negligible IL-8 at 1 hr with a subsequent increase in IL-8 secretion up to 18 hr post stimulation (data not shown). Therefore, we used the 6 hr time point to detect IL-8 in our system in between endemic controls and LL subjects. In addition, this allowed us to compare IL-8 and TNF-α in the same interval.

As we had observed a trend of higher spontaneous secretion of TNF-α in LL-LT group (Fig. 1), we also compared mycobacterium-induced TNF-α and IL-8 activation between the LL-ST and LL-LT groups (data not shown). Neither TNF-α (p = 0.22) nor IL-8 (p = 0.87) secretion between LL-ST and LL-LT groups was found to be significantly different using Students t-test analysis, suggesting that both treatment groups showed similar responses and for the purpose of this study could be studied together.

Both BCG and *M. leprae* were able to induce incremental responses in LL patients over the ongoing IL-8 response (Fig. 2A). There was little increase in IL-8 secretion observed in endemic controls in response to mycobacterium stimulation. The overall IL-8 responses in LL patients were 3 to 5 five-fold higher than in endemic controls. In contrast, monocytes from both endemic controls and lepromatous patients showed significantly greater TNF-α production in response to BCG stimulation as compared with *M. leprae* (Fig. 2B). With, *M. leprae*-induced TNF-α found to be negligible in the LL group. In comparison to the endemic controls and LL groups, BCG-induced TNF-α activation was significantly greater in endemic controls (p = 0.001) than in LL. These results clearly demonstrate that there is an overall increase in IL-8 secretion in LL patients which is independent of TNF-α responses.

**BCG and *M. leprae* induced gene expression in endemic controls and LL subjects.** We next determined cytokine gene expression induced by BCG and *M. leprae*. In addition to IL-8 and TNF-α, we also monitored TGF-β expression, which has previously been shown to be raised in lepromatous infections (10, 15). We first looked at early gene expression at 1 hr post-infection in endemic controls. Spontaneous gene expression of TNF-α and IL-8 was comparable at 1 hr. This was in contrast to the greater TNF-α secretion induced by BCG (Fig. 2). There was some TGF-β mRNA activation present in unstimulated which was transcriptionally upregulated similarly by both BCG and *M. leprae* stimulation.

We subsequently determined a time course of cytokine gene expression profiles in leprosy patients. Spontaneous IL-8 gene expression was detectable within 1 hr and was maintained up to 18 hr, while BCG and
Lepromatous leprosy infection is characterized by the absence of well-defined granuloma formation at the site of infection. Although both macrophages and neutrophils are found at the site of infection, critical cytokines required for granuloma formation (TNF-α and IFN-γ) are absent in these patients (2). Leucocyte chemoattractants such as, IL-8, MCP-1, and MIP-1 are involved in cell recruitment in the early phase of infection and there is evidence that they may be involved in the chronic phase of mycobacterial disease (6, 21). In the current study we show spontaneous mRNA expression of IL-8 and TGF-β from monocytes of LL patients in the absence of TNF-α, suggesting an ongoing in vivo response.

Although our LL patients had been variably treated (0 to 6 months) the mean BI of the LL-ST and LL-LT groups was similar. In addition, the limited chemotherapy used had little effect on ongoing spontaneous IL-8 and TNF-α response, or on the BCG or M. leprae-induced responses as compared between the groups (data not shown). These results led us to consider the LL patients as a single group for this current analysis.

Spontaneous IL-8 in monocytes from lepromatous patients patients has not been previously reported, although IL-8 mRNA has been observed mainly in patients undergoing reactions (26) where it is thought that it may have a role in generating the pathology associated with leprosy reactions. Reduced TNF-α correlates with previous reports of low proinflammatory cytokines found in LL subjects (40). Due to the high basal IL-8 levels in LL patients, it was not surprising to observe little incremental increase in BCG and M. leprae induced IL-8 mRNA expression and secretion. This phenomenon of lowered IL-8 inducibility has been previously described by Friedland, et al. in leucocytes of patients who suffered from fatal tuberculosis and were unable to respond to lipo-polysaccharide (LPS) stimulation by appropriate secretion of TNF-α or IL-8, despite having raised serum levels of IL-8 (6).

In comparison of BCG- and M. leprae-cytokine activation, the latter consistently induced lower IL-8 and TNF-α secretion in both LL and endemic controls groups. The
IL-8 levels we observed in EC are lower than those reported by Mendez-Samperio, et al. (25). Differences in cytokine production can be attributed to mycobacterial strain variations, as we used the BCG Montreal vaccine strain while, Mendez-Samperio, et al. employed the BCG Danish vaccine strain. In addition, immune responses of healthy subjects from mycobacterial endemic regions vary from those of non-endemic regions. In endemic controls, we observed TNF-α secretion in response to BCG but none to M. leprae, correlating with previous reports that M. leprae has been shown to be a poor stimulator of immune activating cytokines IL-1, TNF-α and IL-6 (28, 37). This cannot be attributed to a difference in viability of the two species, as although the M. leprae was γ-irradiated, it has been shown to be a reliable tool as this method of killing is thought to preserve cell wall structure and therefore, antigenicity of the bacillus (1). In addition, previous studies have also shown that M. leprae live and dead induced similar cytokine responses (37).

However, we found M. leprae–induced TNF-α mRNA at 1 hr post-infection M. leprae in endemic controls, which correlates with reports by Shimizu, et al. (34). This discrepancy may possibly be due to post-transcriptional down-regulation of TNF-α by M. leprae. As, M. leprae triggers host cellular signaling pathways tyrosine kinases (22) and mitogen activated protein kinase pathways (11) upon attachment and uptake into cells, which can in turn downregulate TNF-α activation.

The absence of TNF-α in LL at the disease site (2) despite high bacterial load, and in PBMCs from LL patients in response to M. leprae (1), is therefore not surprising. However, the high spontaneous secretion of IL-8 in LL patients is suggests that other factors in vivo may be modulating these monocytes. We observed spontaneous IL-8 gene expression in all of the LL patients studied, which is consistent with the high levels of IL-8 protein secretion detected. A time course gene expression profile in LL patients showed that the relatively low TNF-α stimulation in response to both

| Table 2. Gene expression of IL-8, TNF-α and TGF-β in LL subjects. |
|-----------------|-----------------|-----------------|-----------------|
|                 | IL-8*           | TNF-α*          | TGF-β*          |
|                 | EC   | LL    | EC   | LL    | EC   | LL    |
| Spon.           | 0 (4)| 5 (6)| 0 (4)| 3 (4)| 0 (3)| 4 (4)|
| BCG             | 2 (2)| 4 (4)| 2 (2)| 3 (3)| 2 (2)| 3 (3)|
| M. leprae       | 3 (3)| 4 (4)| 1 (2)| 2 (3)| 2 (2)| 3 (3)|

*Positive samples expressed distinct relevant bands in the PCR reactions; Number of samples positive for gene expression (total number of samples).
BCG and *M. leprae* was not due to suboptimal time of detection in the assay.

Our data indicates that mycobacterium-induced IL-8 activation in leprosy is independent of TNF-α. Previous studies show that TNF-α can enhance IL-8 activity (29, 36), but activation of IL-8 and TNF-α can also occur via different pathways, dependent on the stimulus (35). Our results correlate with reports that *M. tuberculosis*-induced IL-8 secretion is independent of TNF-α (35).

It is the coordinated activation of cytokines that is responsible for the immune profile present in response to mycobacterial infections. TGF-β, is a pleiotropic cytokine important in macrophage immunomodulation and involved in tissue healing and repair (20). Raised TGF-β in leprosy disease especially, in lepromatous infections is thought to be associated with a suppressive action locally (15, 17). TGF-β can inhibit IL-1 and TNF-α secretion in macrophages depending on the stimulus (35). Therefore, the raised constitutive levels of TGF-β we find in the LL subjects may be responsible for the low TNF-α expression observed. In addition, while TGF-β has been shown to downregulate TNF-α induced IL-8 production in endothelial cells (35), it is unable to inhibit *Mycobacterium*-induced IL-8 production (22). Which, may explain the raised IL-8 response despite endogenous TGF-β activation.

Overall, our work shows that while other proinflammatory cytokines may be reduced in the anergic lepromatous patient, the host innate response involving the leucocyte attractant IL-8 is still vigorously ongoing. This may contribute to cell recruitment in severe forms of leprosy even though it may be inappropriate and ineffective and may instead result in pathology rather than protection in the more chronic phase of leprosy infections.

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Leprosy exhibits a wide spectrum of disease presentation varying from the tuberculoid to the lepromatous type of leprosy spectrum depending upon the immune status of the individual (1). Lepromatous (LL) leprosy and borderline lepromatous (BL) types of leprosy are generalized forms of the disease with widespread skin infiltration, numerous macules, papules, plaques or nodules distributed symmetrically all over the body (1). Histoid leprosy and lucio leprosy are variants of lepromatous leprosy. Rarely, Lepromatous leprosy patients presenting with a solitary or only a few lesions along with a high bacterial count are reported (1, 2, 6, 7, 9). In this paper we report a patient of borderline lepromatous leprosy presenting with a single lesion on the face and discuss its significance.

CASE REPORT

Single Lesion Borderline Lepromatous Leprosy

Bikash Ranjan Kar, P. R. Belliappa, Gigi Ebenezer, and C. K. Job

ABSTRACT

A patient is reported who presented with a single lesion on the face which, on histopathological examination, was found to be borderline lepromatous leprosy. The importance of doing skin smears as a routine in all patients to differentiate Multibacillary from Paucibacillary disease is emphasized.

RÉSUMÉ

Nous rapportons ici un patient avec une seule lésion sur la face qui, à l’examen histopathologique, fut diagnostiqué comme souffrant de lèpre borderline. Ce cas souligne l’importance de réaliser des frottis de suc dermique en routine chez tous les patients pour différencier la maladie multibacillaire de la maladie paucibacillaire.

RESUMEN

Se informa el caso de un paciente que se presenta con una sola lesión en la cara; el examen histopatológico de la lesión fue indicativo de un caso de lepra lepromatosa subpolar. Se enfatiza la importancia de hacer, como rutina, el estudio de extensiones de linfa cutánea en todos los pacientes con el fin de diferenciar la enfermedad multibacilar de la paucibacilar.

Leprosy exhibits a wide spectrum of disease presentation varying from the tuberculoid to the lepromatous type of leprosy spectrum depending upon the immune status of the individual (1). Lepromatous (LL) leprosy and borderline lepromatous (BL) types of leprosy are generalized forms of the disease with widespread skin infiltration, numerous macules, papules, plaques or nodules distributed symmetrically all over the body (1). Histoid leprosy and lucio leprosy are variants of lepromatous leprosy. Rarely, Lepromatous leprosy patients presenting with a solitary or only a few lesions along with a high bacterial count are reported (1, 2, 6, 7, 9). In this paper we report a patient of borderline lepromatous leprosy presenting with a single lesion on the face and discuss its significance.

CASE HISTORY

A 45-year-old housewife belonging to a moderately endemic leprosy area in Tamil Nadu, India presented with a single erythematous plaque over the bridge of the nose present for 6 months. On examination, the solitary infiltrated erythematous plaque located over the bridge of the nose extended to the forehead, measuring 4 cm × 3 cm, with well to ill-defined borders (Fig. 1). The sensation over the lesion was preserved. No other lesions were present over the rest of the body. There was no peripheral nerve thickening. No history of contact could be elicited. Clinically, the possibilities of borderline tuberculoid leprosy with mild type 1 reaction, lupus vulgaris, and sarcoidosis were considered. Skin smear from the lesion revealed a Bacillary Index (BI) of 3+ while it was negative from the routine sites. Nasal scrapings were negative for Acid Fast Bacillus (AFB). Lepromin test read at 21 days was also negative. All other routine laboratory investigations were within normal limit. The biopsy from the lesion revealed diffuse atrophy of the epidermis. There was a subepidermal free zone. The dermis showed a dense granulomatous infil-
trate around the blood vessels and the pilosebaceous apparatus. The inflammatory infiltrate was composed of sheets of macrophages and lymphocytes (Fig. 2). The dermal nerves showed intraneural lymphocytic infiltrate. The upper dermal blood vessels were dilated and there was edema. The Granuloma Fraction was 80%. Stain for AFB showed clumps of bacilli within the granulomas. Some of them were solidly staining. Bacillary index of the granuloma (BIG) was 4+.

Based on the smear results and histopathological report the patient was classified as borderline lepromatous (BL) and was started on World Health Organization (WHO) multidrug therapy (MDT) regimen for multibacillary (MB) leprosy.

DISCUSSION
Clinically, this case appeared as borderline tuberculoid (BT) leprosy with mild type 1 reaction. But the skin smear examination from the lesion showed a moderately high bacterial index and the histopathological examination confirmed that this was a lesion of borderline lepromatous leprosy. This clinical and histopathological discrepancy has been reported earlier (2, 8). This case emphasizes the importance of skin smears and the histopathological identification and classification of all patients with unusual lesions. Without such information, this patient would have been considered tuberculoid or borderline tuberculoid leprosy and would have received treatment designed for paucibacillary (PB) patients, which may not have been adequate. It is not always routinely possible to do a histopathological examination but skin smear from selective sites, which is a relatively minor procedure, should always be done in patients with one or few skin patches to help in differentiating MB from PB cases. This is all the more important in a period when the disease is under control, and patients report with early lesions and advanced disease has become a rarity. To our knowledge this is the first case of localized borderline lepromatous (BL) leprosy to present on the face, whereas reports of localized lepromatous leprosy involving the extremities have been published earlier (6, 7).

The localization of the lesion to the glabellar portion of the forehead also merits attention. Mycobacterium leprae is known to enter the body of a susceptible host through abrasions following minor trauma (6). There have been reports of the development of leprosy at the sites of accidental inoculation, thorn pricks (5) and tattooing (7). The patient in our study had been using sindoor (a pigment used for beautification) over the central part of forehead over many years, and it is possible that this could be the initiating event. Though no history of allergy to the pigment used could be elicited, the hypopigmentation present at the site of the application may be an outcome of ongoing subacute inflammation. The possibility that leprosy bacilli might have contaminated the pigment used by the patient should also be seriously considered.
REFERENCES

Earlier trials of BCG vaccine in the protection against leprosy addressed the question of whether or not BCG was effective against leprosy. BCG vaccine trials against leprosy in South America, Africa, and Asia have demonstrated that the BCG vaccine protects against leprosy (4). However the degree of protection conferred by BCG is variable between different populations as is its efficacy in the prevention of tuberculosis (6). Repeated doses of BCG confer additional protection against leprosy, but probably not against tuberculosis (3, 5).

The issue now is not whether BCG is effective but rather what is the best way to use BCG to protect against leprosy? Who should be vaccinated, when should they be vaccinated and how often? In this issue of the INTERNATIONAL JOURNAL OF LEPROSY, Sergio Cunha and colleagues describe a trial to compare two different BCG vaccination strategies. The one currently recommended in Brazil which is population neonatal BCG vaccination and vaccination of household contacts of leprosy patients, versus population neonatal BCG vaccination and vaccination of all school children aged 7 to 14 years.

Neonatal BCG vaccination is recommended in Brazil to protect against tuberculosis. The fact that BCG also protects against leprosy is a bonus. Indeed the degree of protection against leprosy may be greater than that conferred against tuberculosis (6). It would be difficult to justify the use of BCG at a population level on the basis of protection against leprosy alone because of the very low incidence rates of leprosy. The leprosy community is very supportive (7) of the continued use of BCG in leprosy endemic countries, particularly when the costs of the BCG vaccination program are not charged against the limited leprosy budget. The widespread use of BCG vaccine with high population coverage is considered to be a very important factor in the decline in the new case detection rates of leprosy observed in many countries. It is also estimated that the continued use of BCG will be a critical factor affecting the long term trends in incidence rate of leprosy (6).

The lack of a consistent protective effect of BCG re-vaccination against tuberculosis makes the policy of routine BCG re-vaccination in the whole population less economically viable. The most recent World Health Organization Expert Committee on Leprosy (10) did not recommend routine repeated doses of BCG to prevent leprosy because of poor cost-effectiveness, lack of acceptability to recipients, operational difficulties, and the fact that the vaccine (BCG is a live vaccine) is contra-indicated in patients showing symptoms of HIV infection.

In Brazil, neonatal BCG vaccination and selective BCG re-vaccination of household contacts of leprosy patients is recommended. The trial described by Sergio Cunha in this issue sets out to compare this current BCG strategy with an approach where all school age children are re-vaccinated. Similar research questions are being explored in the use of chemoprophylaxis (1); should chemoprophylaxis be give to whole communities or selectively to household contacts (7). A meta-analysis of chemoprophylaxis trials suggests that community coverage has greater efficacy but that selective household contact strategies are more cost effective (7). Analysis of the numbers needed to vaccination to prevent one case gives a simple esti-
mate of the relative cost effectiveness of the different regimens. Household contacts are at higher risk of leprosy than the general population, but only a minority of new cases are from household contacts. Selective high risk approaches are more cost effective than population strategies, but they fail to prevent the majority of new cases. Clearly exposure to *M. leprae* occurs outside the household as well as within households, although the relative importance of household contacts compared with non-household contacts may vary between high and low endemic countries. It is very important that the analysis of this trial in Brazil includes economic appraisal as well as simply measuring vaccine effectiveness. The economic analysis is vital to inform future policy development. It is important to know not just about the effectiveness of the vaccine strategy but also, where resources are scarce, about its cost effectiveness.

This trial described in the journal uses a cluster randomised allocation to intervention groups rather than individual random allocation. This is a robust study design and is appropriate for evaluating public health interventions. The use of clusters as opposed to individuals influences the sample size calculation and this is discussed in the paper. This design also has implications for the analysis and will need to be taken into consideration in the presentation and the interpretation of the results; one important effect is on the size of the confidence intervals.

Tuberculosis is important for leprosy. It is unlikely that this Brazil trial would be taking place for leprosy alone. This is true of many other BCG studies and indeed for many aspects of leprosy research. There was a time when moving from leprosy research to tuberculosis was seen almost as an act of treason, now interchange between leprosy and tuberculosis research is essential. It is important that all future vaccine development for tuberculosis considers the impact on leprosy.

This Brazil trial illustrates the renewed interest in investigating strategies to prevent leprosy and in research that addresses transmission and incidence of leprosy. Other examples of this renewed interest are seen in the focus on chemoprophylaxis and development of new diagnostic tests. Multi-drug therapy (MDT) has had a very dramatic and global impact over the last decade in reducing the prevalence of leprosy. However, there has not been the same impact on new case detection rate. This has provoked renewed research interest in transmission, prevention and early diagnosis. This trial is a good example of the renewed commitment of the research community to explore approaches to the eradication of leprosy.

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CORRESPONDENCE

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

Active Surveillance of Leprosy Contacts in Country with Low Prevalence Rate

ABSTRACT

For advanced control of leprosy in Pakistan where the World Health Organization leprosy elimination goal was achieved in 1996, we conducted surveillance of Mycobacterium leprae-seropositive patients and their contacts and drug resistant strains of M. leprae.

We measured anti-PGL-I antibody level in sera from leprosy patients and their contacts for early detection of M. leprae infection. Out of 34 leprosy patients undergoing treatment, 4 lepromatous leprosy patients were antibody positive, and 6.8 to 23.7 percent of occupational or household contacts were seropositive. Furthermore, three cases (1.2%) had a high antibody titer. For surveillance of drug resistant strains of M. leprae, dapsone and rifampin were targeted. Four out of 18 polymerase chain reaction (PCR) positive samples had mutation in folP gene, and among 10 PCR positive samples, one had a mutation in the rpoB gene.

These results indicate that serological analysis of patient contacts might be useful to find out high risk individuals, and there are M. leprae strains resistant to chemotherapeutic agents in Pakistan.

RÉSUMÉ

Dans le cadre du contrôle avancé de la lèpre au Pakistan où le programme de l’Organisation Mondiale de la Santé a atteint son but d’élimination en 1996, nous avons mené une étude d’épidémio-surveillance des patients séropositifs contre Mycobactérium leprae, de leurs contacts et des souches résistantes de M. leprae aux médicaments.

Nous avons mesuré les niveaux d’anticorps anti-PGL-I dans le sérum de patients lépreux et des personnes en contact avec ces derniers afin d’effectuer une détection précoce de l’infection par M. leprae. Parmi 34 patients actuellement sous traitement, 4 patients lépromateux étaient positifs à l’examen sérologique, et 6,8 à 23,7 pour cent des personnes en contact, soit professionnel, soit domestiques, furent séropositifs. De plus, 3 cas (1,2%) présentaient un titre élevé. La résistance à la dapsone et la rifampicine furent évaluées pour la surveillance des souches résistantes de M. leprae. Quatre des 18 échantillons positifs par PCR présentaient des mutations du gène folP et, parmi 10 échantillons positifs par PCR, une avait une mutation du gène rpoB.

Ces résultats indiquent que l’analyse sérologique des contacts proches de patients hanseniens pourrait bien être utile pour découvrir les individus à haut risque et qu’il existe des souches de M. leprae résistantes aux médicaments chimiothérapeutiques au Pakistan.

RESUMEN

Se hizo un estudio en Pakistán, donde la meta de la OMS de eliminación de la lepra se logró en 1996, para evaluar la evolución de los pacientes sero-positivos a Mycobacterium leprae y sus contactos, y para detectar cepas de M. leprae resistentes a las drogas antileprosas.

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TO THE EDITOR:

In Pakistan, the multi-drug therapy (MDT) program against leprosy conducted by the World Health Organization (WHO) to eliminate the disease was quite successful, and the present prevalence rate is 0.1 per 10,000 inhabitants. However, there are “hot spot areas” where the prevalence rates are still as high as 3.4 per 10,000. Although a significant reduction of the total number of cases registered was observed, no apparent reduction of new cases was achieved (9), and the WHO has now recognized a necessity of a serious concern for leprosy control. One of the ways to achieve disease elimination is an active epidemiological surveillance of patient contacts in highly endemic “hot spot areas,” which will be directly associated with detection of leprosy patients at an early stage.

On the other hand, although MDT was designed to prevent the emergence and spread of drug resistant strains, resistant *Mycobacterium leprae* strain have emerged. A strain showing resistance to both dapsone and rifampin was reported in 1993 (3) and, at present, there are even reports indicating the emergence of a strain resistant to multiple drugs (9). These drug resistant strains provide another serious problem and should not be ignored, especially in countries where the leprosy elimination goal has been achieved. Therefore, the development of a useful tool for early detection of leprosy and drug resistant strains is necessary for the prompt initiation of better medication.

In this study, we conducted serological surveillance of household and occupational contacts, and detected drug resistant strains in Karachi, a representative endemic area in Pakistan.

**Serological test for leprosy.** A total of 300 sera from various individuals, including in-and-out patient of CDGK Leprosy hospital, were obtained with informed consent. These sera were donated by 34 leprosy patients under treatment, 193 household contacts, 59 occupational contacts, and 14 non-contact healthy individuals living in Karachi (Table 1). Infection with *M. leprae* was assessed by using SERODIA®-leprae kit (Fuji Rebio Inc., Tokyo, Japan), which detects antibody against phenolic glycolipid-I (PGL-I) (1). Four leprosy patients under treatment were still found to be anti-PGL-I antibody positive (Table 1), and they were

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*Detection of anti-PGL-I antibodies in serially diluted sera by ELISA using NT-P-BSA antigen coated gelatin particles.*

Serum dilution of more than 1:32 showing agglutination was taken as positive.
all lepromatous leprosy patients. However, borderline or tuberculoid leprosy patients had no antibodies against PGL-I. We then examined 193 household and 59 occupational contacts. Among household contacts, 11.5% of children had the antibody as did 6.8% of adult contacts (Table 1). Furthermore, 23.7% of occupational contacts had the antibody. Three out of 14 non-contacts were antibody positive. Further studies should be conducted with a larger number of non-contacts, but presently, we could not obtain informed consent from them. The titers among child contacts and occupational contacts are surprisingly high, which may indicate that some individuals were exposed to \textit{M. leprae}. This is in accordance with a report that the seroprevalence rate was 26 to 28% in the high endemic area, and 7% in the low endemic area in Sulawesi, Indonesia (7). When we measured the antibody in a semi-quantitative fashion, individuals having high antibody titer were found in household and occupational contacts. The titers of antibody varied from low (1:32) to high (1:>512) values. Three cases out of 252 (1.2%) samples showed quite high (1:>512) antibody titer. These individuals should have a clinical examination to monitor the leprosy manifestation. It has been reported that anti-PGL-I antibody level can reflect the disease activity (6). Therefore, it might be reasonable to speculate that the antibody production was suppressed by successful MDT treatment.

**Detection of drug resistant \textit{Mycobacterium leprae}.** Multi-bacillary (MB) type leprosy patients, either under or after MDT treatment, were targeted to obtain bacilli in the biopsy specimen. \textit{M. leprae} genomic DNA was extracted from the specimens as described previously (5).

To detect drug resistant \textit{M. leprae}, based on the previous studies (4, 6, 8), we targeted mutations of the \textit{folP} gene encoding dihydropteroate synthase (DHPS) for dapsone (5), and the \textit{rpoB} gene for rifampin resistance (4, 8). The polymerase chain reaction (PCR) conditions and primers for \textit{folP} and \textit{rpoB} are as described previously (5, 6). The amplified products from each primer pair were sequenced by using the ABI Prism 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Norwalk, CT, U.S.A.).

Thirty-nine skin samples were taken from leprosy patients in endemic areas of Pakistan such as Karachi, Peshawar, and Balakot, to detect gene mutations relating to drug resistance (Table 2). The number of samples successfully amplified using primers for \textit{folP} gene from 39 biopsy specimens was 18. Among amplified samples, four samples showed \textit{folP} mutations (22.2%). The \textit{folP} gene mutations were found at position 158th (the numbering system following that of reference 5) in three samples, and position 164th in one sample. These mutations induce amino acid changes from threonine to isoleucine at position 53rd of DHPS and from proline to arginine at 55th, respectively (not shown). These mutations have most commonly been observed in dapsone resistant strains (5). Although a larger number of samples should be analyzed, these observations may indicate that there are dapsone-resistant \textit{M. leprae} in Pakistan. In contrast to \textit{folP} gene, primer pair for \textit{rpoB} less frequently amplified the DNA. The possible reason for the failure might be the presence of less than detectable level of \textit{M. leprae} bacilli. In our hands, the detection limit is approximately ten bacilli per biopsy sample. Also the different amplification efficiency between \textit{folP}

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*Drug resistance related-genes, \textit{folP} and \textit{rpoB} were amplified by PCR, sequenced, and compared with control \textit{M. leprae} strain, Thai 53.

†Number of samples successfully amplified by PCR.
and rpoB might depend on a difference of the specificity of primers for each gene. Among ten rpoB gene samples amplified from the 39 biopsies, one sample showed the gene mutation at position 550th of the M. leprae β subunit gene of RNA polymerase. This position was not a so-called “hot spot” of rpoB-associated resistant mutations; however, it induced a change of amino acid residue from aspartic acid to glycine (not shown). There was no relationship among the resistant samples, and no double mutation encoding both folP and rpoB genes was observed.

It is not easy to determine whether the resistant strain developed before or after introduction of MDT. However, there might be some patients who are inadequately treated with MDT due to economical or other social reasons. These patients have a higher risk to produce multidrug-resistant strain than patients adequately treated. Active surveillance is required for control of the spread of drug resistant M. leprae.

Taken together, we showed that some leprosy patient contacts have been infected with M. leprae. Also, dapsone resistance has been detected in Pakistan.

Acknowledgment. We thank Dr. Akira Kobayashi and Dr. Tetsu Nakamura (Peshawar-Kai Hospital, Peshawar, Pakistan) for supplying clinical samples. This work was supported in part by a Health Science Research Grants-Research on Emerging and Re-emerging Infectious Diseases, Ministry of Health, Labour and Welfare, Japan.

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REFERENCES


Phimosis as a Presenting Manifestation of Genital Involvement in Leprosy

TO THE EDITOR:

Though testicular involvement is well known in leprosy, there are only few published reports on the presence of lesions of leprosy on male genital skin. In most of the cases reported, lesions were present on the scrotum with or without involvement of penile shaft, prepuce or glans. We report herein, a case of borderline lepromatous (BL) leprosy in type 1 reaction with cutaneous lesion over the prepuce presenting as phimosis.

CASE REPORT

A 20-year-old male patient presented to us with complaints of erythematous painful swelling of the prepuce with inability to retract for the duration of 1 week. Patient had noticed asymptomatic hypopigmented patch over the same site since 6 to 8 months for which he did not take any treatment. Apart from this, he was not aware of any lesion over other parts of the body prior to this episode. On examination, there was an erythematous, tender, plaque present circumferentially over the prepuce resulting in inability to retract prepuce. Further examination revealed multiple (25 to 30) erythematous hypoesthetic plaques with loss of loss of hair and minimal scaling, measuring from 1 to 5 cm in size over trunk, limbs and one small lesion over the scrotum. His right ulnar nerve, greater auricular nerve, and left common peroneal nerves were thickened with no sensory or motor deficit in the area of their distribution. There was mild tenderness involving right ulnar nerve alone. On slit skin smear examination from ear lobes and lesions (4 sites), BI was 1+ and skin biopsy from one of the lesions showed histopathological features consistent with BL Hansen. Patient was started on World Health Organization (WHO) multi-drug therapy (MDT) multibacillary (MB) regimen and Tab. prednisolone 30 mg daily. Within a week of starting treatment, swelling and tenderness regressed almost completely and there was no difficulty in retraction of prepuce. Prednisolone was gradually tapered over the next 12 weeks and patient has continued to do well at six months follow-up.

DISCUSSION

Although no part of the skin is immune from invasion by *Mycobacterium leprae* (7), the genital skin has been described as an unusual site for leprosy (1). Genital skin has been reported to be relatively cooler than the core temperature under experimental conditions, and thus expected to be at increased risk of infiltration by *Mycobacterium leprae* (4). However, due to the use of occlusive undergarments, it is likely that the temperature of the genital skin may not remain that low and this elevated temperature may possibly make this area less prone to the development of leprosy lesions (5).

Clinical involvement of the genital skin in leprosy has not been studied widely, largely because of the inability in examining patients in totality in routine clinical set-up. Fox and Knott (3) first time reported involvement of male genitals in the form of leprous nodules on the scrotum, prepuce and glans. Parikh, et al. (9) reported six cases of borderline leprosy with lesions on scrotum and penis. Dixit, et al. (2) reported presence of scrotal lesions in tuberculoid leprosy. Kumar, et al. (6) observed genital lesions in 6.6% of all male cases of leprosy. They were seen most frequently in leproma-
tous leprosy (25.8%) followed by borderline lepromatous (13.3%) and borderline tuberculoid (1.4%) leprosy. Arora, et al. (1) found genital lesions in 2.9% of the cases with borderline disease. Most of their patients belonged to the borderline group and were in type 1 reaction. Rarely, histoid lesions have also been reported on the male genitals (7, 10).

*Mycobacterium leprae* has been found in the dartos muscle of scrotum even after adequate therapy (11). Pandya and Anita (8) have reported leprous granulomas and AFB in one third of biopsies from the scrotal skin in patients with all types of leprosy even in the absence of lesions on scrotum. Our case presenting with leprosy lesion on prepuce as phimosis is probably first of its kind. Recently published reports (6, 12) indicate that genital skin lesions in male leprosy patients are not as uncommon as suggested previously. These lesions are missed either because they are not looked for carefully or reluctance on the part of patients to expose the genitals. Therefore, genital examination of leprosy patients is important not only to document its involvement but also to find out any other associated disease, which will require more attention than mere documentation.

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Lepromatous Leprosy with Extensive Unusual Ulcerations and Cachexia. Is It the First Case of Lucio’s Phenomenon from Iran?1

ABSTRACT

We report a 33-year-old Iranian woman with widespread ulcerative lesions in the setting of lepromatous leprosy. We think that the sudden appearance of the characteristic necrotic lesions in the absence of fever and other systemic manifestation, and in accordance with epidermal necrosis and the presence of large numbers of AFB in the endothelium are all in favor of the diagnosis of Lucio’s phenomenon for this patient. To our knowledge this is the first patient who may have had this phenomenon reported from the Middle East.

RÉSUMÉ

Nous rapportons ici le cas d’une femme iranienne de 33 ans qui a présenté des lésions disséminées d’ulcérations cutanées dans le contexte d’une lépre lépromateuse. Nous pensons que la soudaine apparition de lésions nécrotiques caractéristiques, en l’absence de fièvre et d’autres manifestations systémiques, et en tenant compte de la nécrose épidermique et de la présence de très nombreux bacilles AAR dans l’endothélium, est en faveur du diagnostic de phénomène de Lucio pour cette patiente. A notre connaissance, il s’agit du premier cas rapporté au Moyen-Orient qui a probablement été victime de ce phénomène.

RESUMEN

Presentamos el caso de una mujer iraní de 33 años con lesiones ulcerativas diseminadas asociadas a lepra lepromatosa. Pensamos que la aparición súbita de las lesiones necróticas características, la ausencia de fiebre y otras manifestaciones sistémicas, la necrosis epidermática y la presencia de grandes números de bacilos ácido-resistentes, indican un caso de fenómeno de Lucio en esta paciente. Hasta donde sabemos, este es el primer caso de un paciente con fenómeno de Lucio en el Medio Este.

TO THE EDITOR:

Northern Iran was an endemic area for leprosy until a few years ago and almost all of the clinical variants of leprosy have been seen in this area. We report here a case of lepromatous leprosy with unusual extensive ulcerations that we think might be the first case of Lucio’s phenomenon from the Middle East.

A 33-year-old woman with a long history of skin lesions was referred to our service after her lesions had started to become generalized and necrotic, a few days previous. She was inhabitant of a far located rural area. Her father was a known case of lepromatous leprosy and had been treated with dapsone as monotherapy during 1970’s and later with standard multiple drug therapy (MDT) for leprosy, but the patient had never visited a dermatologist nor received any antileprosy medications.

On admission to hospital, the patient was cachectic and in poor general condition. An obvious leonine face with nearly complete loss of eyebrows and eyelashes, and extensive ecchymotic patches in association with deep ulcerative lesions were seen on her body, especially on the extremities (Figs. 1 and 2). On laboratory examinations, a full blood count showed anemia with Hb 4.8 g/dl. The white cell count was normal, but platelet count was raised to 7 × 10^5/dl. The erythrocyte sedimentation rate was 72 mm/hr. Rheumatic factor and C-Reactive Protein were weekly positive but other laboratory tests including, renal function tests, liver enzymes, electrolytes, and coagulation tests were all in the normal range.

Slit skin smears from different locations showed multiple acid-fast bacilli (AFB),

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E-mail: ozargari@iranderma.com
and a skin biopsy from an ecchymotic patch revealed swollen endothelial cells with fibrinoid necrosis and a mixed inflammatory infiltrate with nuclear dusts and an atrophic epidermis with focal necrosis. In the dermis, enormous numbers of AFB were seen with Ziehl-Neelsen staining. AFB were also present in the form of clumps in the vessel walls. We started supportive care and a standard antileprosy therapy including dapsona 100 mg daily, clofazimine 50 mg daily and rifampicin 600 mg monthly for the patient. A combination of systemic antibiotics including a 3rd generation cephalosporin and vancomycin was also started after the patient became febrile. During the admission, the patient’s hemoglobin dropped and she developed hematuria and pancytopenia and unfortunately died at the 35th day of admission because of severe sepsis.

Lucio’s phenomenon is a severe necrotizing reaction occurring in the diffuse, lepromatous leprosy. Lucio and Alvarado first described this phenomenon in 1852. Latapi and
Chevez later denoted this reaction in 1948 as Lucio’s phenomenon (3). This type of reaction is most commonly seen in Mexico and Central America (3, 6, 8), and is rare outside America, although Saoji, et al. reported two cases of this phenomenon from India (9), and Ang, et al. recently reported two Chinese men with fatal Lucio’s phenomenon (1).

Painful macules or plaques progressing to ulcers characterize Lucio’s phenomenon. Features of the underlying lepromatous leprosy commonly described include diffuse thickening of facial skin, maderosis, and destructive rhinitis (6). Anemia, lymphadenopathy, splenomegaly, hypoalbuminemia, hypocalcemia and polyclonal gammopathy are among the other reported manifestations (4, 6).

Rea, et al. have hypothesized that patients with Lucio’s phenomenon have an exceptionally deficient defense mechanism, allowing unrestricted proliferation of AFB in endothelial cells, facilitating contact between bacterial antigens and circulating antibody and leading to infarction. Also, this nadir of resistance allows unimpeded dissemination of AFB, accounting for the clinical features of diffuse non-nodular leprosy (7). Most cases of Lucio’s phenomenon have been reported to have a leukocytoclastic vasculitis as the underlying pathologic abnormality, although some cases of Lucio’s phenomenon may be caused by vascular damage due to direct invasion of Mycobacterium leprae and not necessarily by leukocytoclastic vasculitis (8). Deposits of mixed-type cryoglobulins

Fig. 3. Swollen endothelial cells with fibrinoid necrosis and a mixed inflammatory infiltrate (Hematoxylin-Eosin ×100).

Fig. 4. Aggregates of acid-fast bacilli in the endothelial walls (Ziehl-Neelsen ×400).
(IgG, IgA, IgM, C3, and C1q) have been observed in dermis vessels affected by vasculitis of the Lucio’s phenomenon type, suggesting a mechanism mediated by deposits of immune complexes (5).

Lucio’s phenomenon occurs in patients with undiagnosed and untreated leprosy, whereas the erythema nodosum leprosum (ENL) may occur in any type of lepromatous leprosy, and frequently occurs after starting the treatment. The main clinical differences between the Lucio’s phenomenon and ENL are that the former is an ulcerative reaction occurring in the absence of cutaneous nodules, whereas the latter usually present as tender cutaneous nodules that rarely ulcerate (1). Absence of fever, leukocytosis, tenderness, and other systemic presentations such as arthritis, neuritis, and iridocyclitis, and failure to respond to thalidomide are among the other distinct features of Lucio’s phenomenon (6,10). Histologically, Lucio’s reaction can be distinguished from ENL by epidermal necrosis and by necrotizing vasculitis manifesting necrosis in the walls of superficial vessels and severe, focal endothelial proliferation of mid-dermal vessels. Furthermore, the numbers of AFB are much more in the lesions of Lucio’s phenomenon in comparison with The ENL (7). In addition to ENL, in the differential diagnosis of Lucio’s phenomenon, vasculitis of other origins should be considered, such as the antiphospholipid antibody thrombotic syndrome associated with lepromatous leprosy (3).

Although we were not able to assay cryoglobulins, cryofibrinogens, and antiphospholipid antibodies in this patient, normal coagulation tests including partial thromboplastin time make this syndrome improbable for this case.

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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.

Active Surveillance of Leprosy Contacts in Country with Low Prevalence Rate

ABSTRACT

For advanced control of leprosy in Pakistan where the World Health Organization leprosy elimination goal was achieved in 1996, we conducted surveillance of Mycobacterium leprae-seropositive patients and their contacts and drug resistant strains of M. leprae.

We measured anti-PGL-I antibody level in sera from leprosy patients and their contacts for early detection of M. leprae infection. Out of 34 leprosy patients undergoing treatment, 4 lepromatous leprosy patients were antibody positive, and 6.8 to 23.7 percent of occupational or household contacts were seropositive. Furthermore, three cases (1.2%) had a high antibody titer. For surveillance of drug resistant strains of M. leprae, dapsone and rifampin were targeted. Four out of 18 polymerase chain reaction (PCR) positive samples had mutation in folP gene, and among 10 PCR positive samples, one had a mutation in the rpoB gene.

These results indicate that serological analysis of patient contacts might be useful to find out high risk individuals, and there are M. leprae strains resistant to chemotherapeutic agents in Pakistan.

RÉSUMÉ

Dans le cadre du contrôle avancé de la lèpre au Pakistan où le programme de l’Organisation Mondiale de la Santé a atteint son but d’élimination en 1996, nous avons mené une étude d’épidémio-surveillance des patients séropositifs contre Mycobactérium leprae, de leurs contacts et des souches résistantes de M. leprae aux médicaments.

Nous avons mesuré les niveaux d’anticorps anti-PGL-I dans le sérum de patients lépreux et des personnes en contact avec ces derniers afin d’effectuer une détection précoce de l’infection par M. leprae. Parmi 34 patients actuellement sous traitement, 4 patients lépromateux étaient positifs à l’examen sérologique, et 6,8 à 23,7 pour cent des personnes en contact, soit professionnel, soit domestiques, furent séropositifs. De plus, 3 cas (1,2%) présentaient un titre élevé. La résistance à la dapsone et la rifampicine furent évaluées pour la surveillance des souches résistantes de M. leprae. Quatre des 18 échantillons positifs par PCR présentaient des mutations du gène folP et, parmi 10 échantillons positifs par PCR, une avait une mutation du gène rpoB.

Ces résultats indiquent que l’analyse sérologique des contacts proches de patients hanséniens pourrait bien être utile pour découvrir les individus à haut risque et qu’il existe des souches de M. leprae résistantes aux médicaments chimiothérapeutiques au Pakistan.

RESUMEN

Se hizo un estudio en Pakistán, donde la meta de la OMS de eliminación de la lepra se logró en 1996, para evaluar la evolución de los pacientes sero-positivos a Mycobacterium leprae y sus contactos, y para detectar cepas de M. leprae resistentes a las drogas antileprosas.
TO THE EDITOR:

In Pakistan, the multi-drug therapy (MDT) program against leprosy conducted by the World Health Organization (WHO) to eliminate the disease was quite successful, and the present prevalence rate is 0.1 per 10,000 inhabitants. However, there are “hot spot areas” where the prevalence rates are still as high as 3.4 per 10,000. Although a significant reduction of the total number of cases registered was observed, no apparent reduction of new cases was achieved (9), and the WHO has now recognized a necessity of a serious concern for leprosy control. One of the ways to achieve disease elimination is an active epidemiological surveillance of patient contacts in highly endemic “hot spot areas,” which will be directly associated with detection of leprosy patients at an early stage.

On the other hand, although MDT was designed to prevent the emergence and spread of drug resistant strains, resistant Mycobacterium leprae strain have emerged. A strain showing resistance to both dapsone and rifampin was reported in 1993 (3) and, at present, there are even reports indicating the emergence of a strain resistant to multiple drugs (4). These drug resistant strains provide another serious problem and should not be ignored, especially in countries where the leprosy elimination goal has been achieved. Therefore, the development of a useful tool for early detection of leprosy and drug resistant strains is necessary for the prompt initiation of better medication.

In this study, we conducted serological surveillance of household and occupational contacts, and detected drug resistant strains in Karachi, a representative endemic area in Pakistan.

Serological test for leprosy. A total of 300 sera from various individuals, including in-and-out patient of CDGK Leprosy hospital, were obtained with informed consent. These sera were donated by 34 leprosy patients under treatment, 193 household contacts, 59 occupational contacts, and 14 non-contact healthy individuals living in Karachi (Table 1). Infection with M. leprae was assessed by using SERODIA®-leprae kit (Fuji Rebio Inc., Tokyo, Japan), which detects antibody against phenolic glycolipid-I (PGL-I) (1). Four leprosy patients under treatment were still found to be anti-PGL-I antibody positive (Table 1), and they were

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<tr>
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</table>

†Detection of anti-PGL-I antibodies in serially diluted sera by ELISA using NT-P-BSA antigen coated gelatin particles.

Serum dilution of more than 1:32 showing agglutination was taken as positive.
all lepromatous leprosy patients. However, borderline or tuberculoid leprosy patients had no antibodies against PGL-I. We then examined 193 household and 59 occupational contacts. Among household contacts, 11.5% of children had the antibody as did 6.8% of adult contacts (Table 1). Furthermore, 23.7% of occupational contacts had the antibody. Three out of 14 non-contacts were antibody positive. Further studies should be conducted with a larger number of non-contacts, but presently, we could not obtain informed consent from them. The titers among child contacts and occupational contacts are surprisingly high, which may indicate that some individuals were exposed to M. leprae. This is in accordance with a report that the seroprevalence rate was 26 to 28% in the high endemic area, and 7% in the low endemic area in Sulawesi, Indonesia (7). When we measured the antibody in a semi-quantitative fashion, individuals having high antibody titer were found in household and occupational contacts. The titers of antibody varied from low (1:32) to high (1:/>512) values. Three cases out of 252 (1.2%) samples showed quite high (1:/>512) antibody titer. These individuals should have a clinical examination to monitor the leprosy manifestation. It has been reported that anti-PGL-I antibody level can reflect the disease activity (8). Therefore, it might be reasonable to speculate that the antibody production was suppressed by successful MDT treatment.

Detection of drug resistant Mycobacterium leprae. Multi-bacillary (MB) type leprosy patients, either under or after MDT treatment, were targeted to obtain bacilli in the biopsy specimen. M. leprae genomic DNA was extracted from the specimens as described previously (9).

To detect drug resistant M. leprae, based on the previous studies (4, 6, 8), we targeted mutations of the folP gene encoding dihydropteroate synthase (DHPS) for dapsone (9), and the rpoB gene for rifampin resistance (4, 9). The polymerase chain reaction (PCR) conditions and primers for folP and rpoB are as described previously (5, 6). The amplified products from each primer pair were sequenced by using the ABI Prism 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Norwalk, CT, U.S.A.).

Thirty-nine skin samples were taken from leprosy patients in endemic areas of Pakistan such as Karachi, Peshawar, and Balakot, to detect gene mutations relating to drug resistance (Table 2). The number of samples successfully amplified using primers for folP gene from 39 biopsy specimens was 18. Among amplified samples, four samples showed folP mutations (22.2%). The folP gene mutations were found at position 158th (the numbering system following that of reference 5) in three samples, and position 164th in one sample. These mutations induce amino acid changes from threonine to isoleucine at position 53rd of DHPS and from proline to arginine at 55th, respectively (not shown). These mutations have most commonly been observed in dapsone resistant strains (5). Although a larger number of samples should be analyzed, these observations may indicate that there are dapsone-resistant M. leprae in Pakistan. In contrast to folP gene, primer pair for rpoB less frequently amplified the DNA. The possible reason for the failure might be the presence of less than detectable level of M. leprae bacilli. In our hands, the detection limit is approximately ten bacilli per biopsy sample. Also the different amplification efficiency between folP

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* Drug resistance related-genes, folP and rpoB were amplified by PCR, sequenced, and compared with control M. leprae strain, Thai 53.
† Number of samples successfully amplified by PCR.
and rpoB might depend on a difference of the specificity of primers for each gene. Among ten rpoB gene samples amplified from the 39 biopsies, one sample showed the gene mutation at position 550th of the M. leprae ß subunit gene of RNA polymerase. This position was not a so-called “hot spot” of rpoB-associated resistant mutations (8); however, it induced a change of amino acid residue from aspartic acid to glycine (not shown). There was no relationship among the resistant samples, and no double mutation encoding both folP and rpoB genes was observed.

It is not easy to determine whether the resistant strain developed before or after introduction of MDT. However, there might be some patients who are inadequately treated with MDT due to economical or other social reasons. These patients have a higher risk to produce multidrug-resistant strain than patients adequately treated. Active surveillance is required for control of the spread of drug resistant M. leprae.

Taken together, we showed that some leprosy patient contacts have been infected with M. leprae. Also, dapsone resistance has been detected in Pakistan.

Acknowledgment. We thank Dr. Akira Kobayashi and Dr. Tetsu Nakamura (Peshawar-Kai Hospital, Peshawar, Pakistan) for supplying clinical samples. This work was supported in part by a Health Science Research Grants-Research on Emerging and Re-emerging Infectious Diseases, Ministry of Health, Labour and Welfare, Japan.

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REFERENCES
Phimosis as a Presenting Manifestation of Genital Involvement in Leprosy

TO THE EDITOR:

Though testicular involvement is well known in leprosy, there are only few published reports on the presence of lesions of leprosy on male genital skin. In most of the cases reported, lesions were present on the scrotum with or without involvement of penile shaft, prepuce or glans. We report herein, a case of borderline lepromatous (BL) leprosy in type 1 reaction with cutaneous lesion over the prepuce presenting as phimosis.

CASE REPORT

A 20-year-old male patient presented to us with complaints of erythematous painful swelling of the prepuce with inability to retract for the duration of 1 week. Patient had noticed asymptomatic hypopigmented patch over the same site since 6 to 8 months for which he did not take any treatment. Apart from this, he was not aware of any lesion over other parts of the body prior to this episode. On examination, there was an erythematous, tender, plaque present circumferentially over the prepuce resulting in inability to retract prepuce (The Figure). Further examination revealed multiple (25 to 30) erythematous hypoesthetic plaques with loss of loss of hair and minimal scaling, measuring from 1 to 5 cm in size over trunk, limbs and one small lesion over the scrotum. His right ulnar nerve, greater auricular nerve, and left common peroneal nerves were thickened with no sensory or motor deficit in the area of their distribution. There was mild tenderness involving right ulnar nerve alone. On slit skin smear examination from ear lobes and lesions (4 sites), BI was 1+ and skin biopsy from one of the lesions showed histopathological features consistent with BL Hansen. Patient was started on World Health Organization (WHO) multi-drug therapy (MDT) multidrug (MB) regimen and Tab. prednisolone 30 mg daily. Within a week of starting treatment, swelling and tenderness regressed almost completely and there was no difficulty in retraction of prepuce. Prednisolone was gradually tapered over the next 12 weeks and patient has continued to do well at six months follow-up.

DISCUSSION

Although no part of the skin is immune from invasion by Mycobacterium leprae (⁴), the genital skin has been described as an unusual site for leprosy (⁴). Genital skin has been reported to be relatively cooler than the core temperature under experimental conditions, and thus expected to be at increased risk of infiltration by Mycobacterium leprae (⁴). However, due to the use of occlusive undergarments, it is likely that the temperature of the genital skin may not remain that low and this elevated temperature may possibly make this area less prone to the development of leprosy lesions (⁴).

Clinical involvement of the genital skin in leprosy has not been studied widely, largely because of the inability in examining patients in totality in routine clinical set-up. Fox and Knott (⁴) first time reported involvement of male genitals in the form of leprous nodules on the scrotum, prepuce and glans. Parikh, et al. (⁵) reported six cases of borderline leprosy with lesions on scrotum and penis. Dixit, et al. (⁶) reported presence of scrotal lesions in tuberculoid leprosy. Kumar, et al. (⁷) observed genital lesions in 6.6% of all male cases of leprosy. They were seen most frequently in leproma-
tous leprosy (25.8%) followed by borderline lepromatous (13.3%) and borderline tuberculoid (1.4%) leprosy. Arora, et al. (1) found genital lesions in 2.9% of the cases with borderline disease. Most of their patients belonged to the borderline group and were in type 1 reaction. Rarely, histoid lesions have also been reported on the male genitals (7, 10).

*Mycobacterium leprae* has been found in the dartos muscle of scrotum even after adequate therapy (11). Pandya and Anita (8) have reported leprous granulomas and AFB in one third of biopsies from the scrotal skin in patients with all types of leprosy even in the absence of lesions on scrotum. Our case presenting with leprosy lesion on prepuce as phimosis is probably first of its kind. Recently published reports (6, 12) indicate that genital skin lesions in male leprosy patients are not as uncommon as suggested previously. These lesions are missed either because they are not looked for carefully or reluctance on the part of patients to expose the genitals. Therefore, genital examination of leprosy patients is important not only to document its involvement but also to find out any other associated disease, which will require more attention than mere documentation.

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Lepromatous Leprosy with Extensive Unusual Ulcerations and Cachexia. Is It the First Case of Lucio’s Phenomenon from Iran?1

ABSTRACT

We report a 33-year-old Iranian woman with widespread ulcerative lesions in the setting of lepromatous leprosy. We think that the sudden appearance of the characteristic necrotic lesions in the absence of fever and other systemic manifestation, and in accordance with epidermal necrosis and the presence of large numbers of AFB in the endothelium are all in favor of the diagnosis of Lucio’s phenomenon for this patient. To our knowledge this is the first patient who may have had this phenomenon reported from the Middle East.

RÉSUMÉ

Nous rapportons ici le cas d’une femme iranienne de 33 ans qui a présenté des lésions disséminées d’ulcérations cutanées dans le contexte d’une lèpre lépromateuse. Nous pensons que la soudaine apparition de lésions nécrotiques caractéristiques, en l’absence de fièvre et d’autres manifestations systémiques, et en tenant compte de la nécrose épidermique et de la présence de très nombreux bacilles AAR dans l’endothélium, est en faveur du diagnostic de phénomène de Lucio pour cette patiente. A notre connaissance, il s’agit du premier cas rapporté au Moyen-Orient qui a probablement été victime de ce phénomène.

RESUMEN

Presentamos el caso de una mujer iraní de 33 años con lesiones ulcerativas diseminadas asociadas a lepra lepromatosa. Pensamos que la aparición súbita de las lesiones necróticas, la ausencia de fiebre y otras manifestaciones sistémicas, la necrosis epidérmica y la presencia de grandes números de bacilos ácido-resistentes, indican un caso de fenómeno de Lucio en esta paciente. Hasta donde sabemos, este es el primer caso de un paciente con fenómeno de Lucio en el Medio Este.

TO THE EDITOR:

Northern Iran was an endemic area for leprosy until a few years ago and almost all of the clinical variants of leprosy have been seen in this area. We report here a case of lepromatous leprosy with unusual extensive ulcerations that we think might be the first case of Lucio’s phenomenon from the Middle East.

A 33-year-old woman with a long history of skin lesions was referred to our service after her lesions had started to become generalized and necrotic, a few days previous. She was inhabitant of a far located rural area. Her father was a known case of lepromatous leprosy and had been treated with dapsone as monotherapy during 1970’s and later with standard multiple drug therapy (MDT) for leprosy, but the patient had never visited a dermatologist nor received any antileprosy medications.

On admission to hospital, the patient was cachectic and in poor general condition. An obvious leonine face with nearly complete loss of eyebrows and eyelashes, and extensive ecchymotic patches in association with deep ulcerative lesions were seen on her body, especially on the extremities (Figs. 1 and 2). On laboratory examinations, a full blood count showed anemia with Hb 4.8 g/dl. The white cell count was normal, but platelet count was raised to $7 \times 10^5$/dl. The erythrocyte sedimentation rate was 72 mm/hr. Rheumatic factor and C-Reactive Protein were weekly positive but other laboratory tests including, renal function tests, liver enzymes, electrolytes, and coagulation tests were all in the normal range.

Slit skin smears from different locations showed multiple acid-fast bacilli (AFB),

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and a skin biopsy from an ecchymotic patch revealed swollen endothelial cells with fibrinoid necrosis and a mixed inflammatory infiltrate with nuclear dusts and an atrophic epidermis with focal necrosis. In the dermis, enormous numbers of AFB were seen with Ziehl-Neelsen staining. AFB were also present in the form of clumps in the vessel walls. We started supportive care and a standard antileprosy therapy including dapson 100 mg daily, clofazimine 50 mg daily and rifampicin 600 mg monthly for the patient. A combination of systemic antibiotics including a 3rd generation cephalosporin and vancomycin was also started after the patient became febrile. During the admission, the patient’s hemoglobin dropped and she developed hamaturia and pancytopenia and unfortunately died at the 35th day of admission because of severe sepsis.

Lucio’s phenomenon is a severe necrotizing reaction occurring in the diffuse, lepromatous leprosy. Lucio and Alvarado first described this phenomenon in 1852. Latapi and
Chevez later denoted this reaction in 1948 as Lucio’s phenomenon (3). This type of reaction is most commonly seen in Mexico and Central America (3, 6, 8), and is rare outside America, although Saoji, et al. reported two cases of this phenomenon from India (9), and Ang, et al. recently reported two Chinese men with fatal Lucio’s phenomenon (1).

Painful macules or plaques progressing to ulcers characterize Lucio’s phenomenon. Features of the underlying lepromatous leprosy commonly described include diffuse thickening of facial skin, maderosis, and destructive rhinitis (6). Anemia, lymphadenopathy, splenomegaly, hypoalbuminemia, hypocalcemia and polyclonal gammopathy are among the other reported manifestations (4, 6).

Rea, et al. have hypothesized that patients with Lucio’s phenomenon have an exceptionally deficient defense mechanism, allowing unrestricted proliferation of AFB in endothelial cells, facilitating contact between bacterial antigens and circulating antibody and leading to infarction. Also, this nadir of resistance allows unimpeded dissemination of AFB, accounting for the clinical features of diffuse non-nodular leprosy (7). Most cases of Lucio’s phenomenon have been reported to have a leukocytoclastic vasculitis as the underlying pathologic abnormality, although some cases of Lucio’s phenomenon may be caused by vascular damage due to direct invasion of Mycobacterium leprae and not necessarily by leukocytoclastic vasculitis (4). Deposits of mixed-type cryoglobulins

Fig. 3. Swollen endothelial cells with fibrinoid necrosis and a mixed inflammatory infiltrate (Hematoxylin-Eosin ×100).

Fig. 4. Aggregates of acid-fast bacilli in the endothelial walls (Ziehl-Neelsen ×400).
(IgG, IgA, IgM, C3, and C1q) have been observed in dermis vessels affected by vasculitis of the Lucio’s phenomenon type, suggesting a mechanism mediated by deposits of immune complexes (5).

Lucio’s phenomenon occurs in patients with undiagnosed and untreated leprosy, whereas the erythema nodosum leprosum (ENL) may occur in any type of lepromatous leprosy, and frequently occurs after starting the treatment. The main clinical differences between the Lucio’s phenomenon and ENL are that the former is an ulcerative reaction occurring in the absence of cutaneous nodules, whereas the latter usually present as tender cutaneous nodules that rarely ulcerate (1). Absence of fever, leukocytosis, tenderness, and other systemic presentations such as arthritis, neuritis, and iridocyclitis, and failure to respond to thalidomide are among the other distinct features of Lucio’s phenomenon (6,10). Histologically, Lucio’s reaction can be distinguished from ENL by epidermal necrosis and by necrotizing vasculitis manifesting necrosis in the walls of superficial vessels and severe, focal endothelial proliferation of mid-dermal vessels. Furthermore, the numbers of AFB are much more in the lesions of Lucio’s phenomenon in comparison with The ENL (7). In addition to ENL, in the differential diagnosis of Lucio’s phenomenon, vasculitis of other origins should be considered, such as the antiphospholipid antibody thrombotic syndrome associated with lepromatous leprosy (8).

Although we were not able to assay cryoglobulins, cryofibrinogens, and antiphospholipid antibodies in this patient, normal coagulation tests including partial thromboplastin time make this syndrome improbable for this case.

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—Omid Zargari, M.D.,
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Active Surveillance of Leprosy Contacts in Country with Low Prevalence Rate

ABSTRACT

For advanced control of leprosy in Pakistan where the World Health Organization leprosy elimination goal was achieved in 1996, we conducted surveillance of *Mycobacterium leprae*-seropositive patients and their contacts and drug resistant strains of *M. leprae*.

We measured anti-PGL-I antibody level in sera from leprosy patients and their contacts for early detection of *M. leprae* infection. Out of 34 leprosy patients undergoing treatment, 4 lepromatous leprosy patients were antibody positive, and 6.8 to 23.7 percent of occupational or household contacts were seropositive. Furthermore, three cases (1.2%) had a high antibody titer. For surveillance of drug resistant strains of *M. leprae*, dapsone and rifampin were targeted. Four out of 18 polymerase chain reaction (PCR) positive samples had mutation in *folP* gene, and among 10 PCR positive samples, one had a mutation in the *rpoB* gene.

These results indicate that serological analysis of patient contacts might be useful to find out high risk individuals, and there are *M. leprae* strains resistant to chemotherapeutic agents in Pakistan.

RÉSUMÉ

Dans le cadre du contrôle avancé de la lépre au Pakistan où le programme de l’Organisation Mondiale de la Santé a atteint son but d’élimination en 1996, nous avons mené une étude d’épidémio-surveillance des patients séropositifs contre *Mycobactérium leprae*, de leurs contacts et des souches résistantes de *M. leprae* aux médicaments.

Nous avons mesuré les niveaux d’anticorps anti-PGL-I dans le sérum de patients lépreux et des personnes en contact avec ces derniers afin d’effectuer une détection précoce de l’infection par *M. leprae*. Parmi 34 patients actuellement sous traitement, 4 patients lépromateux étaient positifs à l’examen sérologique, et 6.8 à 23,7 pour cent des personnes en contact, soit professionnel, soit domestiques, furent séropositifs. De plus, 3 cas (1,2%) présentaient un titre élevé. La résistance à la dapsone et la rifampicine furent évaluées pour la surveillance des souches résistantes de *M. leprae*. Quatre des 18 échantillons positifs par PCR présentaient des mutations du gène *folP* et, parmi 10 échantillons positifs par PCR, une avait une mutation du gène *rpoB*.

Ces résultats indiquent que l’analyse sérologique des contacts proches de patients han-séniens pourrait bien être utile pour découvrir les individus à haut risque et qu’il existe des souches de *M. leprae* résistantes aux médicaments chimiothérapeutiques au Pakistan.

RESUMEN

Se hizo un estudio en Pakistán, donde la meta de la OMS de eliminación de la lepra se logró en 1996, para evaluar la evolución de los pacientes sero-positivos a *Mycobacterium leprae* y sus contactos, y para detectar cepas de *M. leprae* resistentes a las drogas antileprosas.
Se midió la presencia de anticuerpos anti-PGL-I en los sueros de los pacientes y sus contactos para detectar la infección temprana por *M. leprae*. De los 34 pacientes en tratamiento, 4 pacientes con lepra lepromatosa (11.7%) tuvieron anticuerpos anti-PGL-I, además de que 6.8% de los contactos ocupacionales y 23.7% de los contactos convivientes también fueron sero-positivos. Tres casos (1.2%) tuvieron anticuerpos anti-PGL-I a títulos elevados. También se estudió la resistencia de las cepas a dapsona y rifampina. Cuatro de 18 muestras positivas por la reacción en cadena de la DNA polimerasa (PCR) tuvieron una mutación en el gene *foIP*, y una de 10 muestras positivas por PCR tuvo una mutación en el gene *rpoB*.

Estos resultados indican que el análisis serológico de los pacientes puede ser útil para detectar a los individuos de alto riesgo, y que en Pakistán hay cepas resistentes a la quimioterapia.

**TO THE EDITOR:**

In Pakistan, the multi-drug therapy (MDT) program against leprosy conducted by the World Health Organization (WHO) to eliminate the disease was quite successful, and the present prevalence rate is 0.1 per 10,000 inhabitants. However, there are “hot spot areas” where the prevalence rates are still as high as 3.4 per 10,000. Although a significant reduction of the total number of cases registered was observed, no apparent reduction of new cases was achieved (9), and the WHO has now recognized a necessity of a serious concern for leprosy control. One of the ways to achieve disease elimination is an active epidemiological surveillance of patient contacts in highly endemic “hot spot areas,” which will be directly associated with detection of leprosy patients at an early stage.

On the other hand, although MDT was designed to prevent the emergence and spread of drug resistant strains, resistant *Mycobacterium leprae* strain have emerged. A strain showing resistance to both dapsona and rifampin was reported in 1993 (3) and, at present, there are even reports indicating the emergence of a strain resistant to multiple drugs (4). These drug resistant strains provide another serious problem and should not be ignored, especially in countries where the leprosy elimination goal has been achieved. Therefore, the development of a useful tool for early detection of leprosy and drug resistant strains is necessary for the prompt initiation of better medication.

In this study, we conducted serological surveillance of household and occupational contacts, and detected drug resistant strains in Karachi, a representative endemic area in Pakistan.

**Serological test for leprosy.** A total of 300 sera from various individuals, including in-and-out patient of CDGK Leprosy hospital, were obtained with informed consent. These sera were donated by 34 leprosy patients under treatment, 193 household contacts, 59 occupational contacts, and 14 non-contact healthy individuals living in Karachi (Table 1). Infection with *M. leprae* was assessed by using SERODIA®-leprae kit (Fuji Rebio Inc., Tokyo, Japan), which detects antibody against phenolic glycolipid-I (PGL-I) (1). Four leprosy patients under treatment were still found to be anti-PGL-I antibody positive (Table 1), and they were

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Detection of anti-PGL-I antibodies in serially diluted sera by ELISA using NT-P-BSA antigen coated gelatin particles.

Serum dilution of more than 1:32 showing agglutination was taken as positive.
all lepromatous leprosy patients. However, borderline or tuberculoid leprosy patients had no antibodies against PGL-I. We then examined 193 household and 59 occupational contacts. Among household contacts, 11.5% of children had the antibody as did 6.8% of adult contacts (Table 1). Furthermore, 23.7% of occupational contacts had the antibody. Three out of 14 non-contacts were antibody positive. Further studies should be conducted with a larger number of non-contacts, but presently, we could not obtain informed consent from them. The titers among child contacts and occupational contacts are surprisingly high, which may indicate that some individuals were exposed to \textit{M. leprae}. This is in accordance with a report that the seroprevalence rate was 26 to 28% in the high endemic area, and 7% in the low endemic area in Sulawesi, Indonesia (7). When we measured the antibody in a semi-quantitative fashion, individuals having high antibody titer were found in household and occupational contacts. The titers of antibody varied from low (1:32) to high (1:>512) values. Three cases out of 252 (1.2%) samples showed quite high (1:>512) antibody titer. These individuals should have a clinical examination to monitor the leprosy manifestation. It has been reported that anti-PGL-I antibody level can reflect the disease activity (5). Therefore, it might be reasonable to speculate that the antibody production was suppressed by successful MDT treatment.

\textbf{Detection of drug resistant \textit{Mycobacterium leprae}.} Multi-bacillary (MB) type leprosy patients, either under or after MDT treatment, were targeted to obtain bacilli in the biopsy specimen. \textit{M. leprae} genomic DNA was extracted from the specimens as described previously (5).

To detect drug resistant \textit{M. leprae}, based on the previous studies (4, 6, 8), we targeted mutations of the \textit{folP} gene encoding dihydropteroate synthase (DHPS) for dapsone (4), and the \textit{rpoB} gene for rifampin resistance (4, 8). The polymerase chain reaction (PCR) conditions and primers for \textit{folP} and \textit{rpoB} are as described previously (5, 6). The amplified products from each primer pair were sequenced by using the ABI Prism 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems, Norwalk, CT, U.S.A.).

Thirty-nine skin samples were taken from leprosy patients in endemic areas of Pakistan such as Karachi, Peshawar, and Balakot, to detect gene mutations relating to drug resistance (Table 2). The number of samples successfully amplified using primers for \textit{folP} gene from 39 biopsy specimens was 18. Among amplified samples, four samples showed \textit{folP} mutations (22.2%). The \textit{folP} gene mutations were found at position 158th (the numbering system following that of reference 5) in three samples, and position 164th in one sample. These mutations induce amino acid changes from threonine to isoleucine at position 53rd of DHPS and from proline to arginine at 55th, respectively (not shown). These mutations have most commonly been observed in dapsone resistant strains (5). Although a larger number of samples should be analyzed, these observations may indicate that there are dapsone-resistant \textit{M. leprae} in Pakistan. In contrast to \textit{folP} gene, primer pair for \textit{rpoB} less frequently amplified the DNA. The possible reason for the failure might be the presence of less than detectable level of \textit{M. leprae} bacilli. In our hands, the detection limit is approximately ten bacilli per biopsy sample. Also the different amplification efficiency between \textit{folP}
and rpoB might depend on a difference of the specificity of primers for each gene. Among ten rpoB gene samples amplified from the 39 biopsies, one sample showed the gene mutation at position 550th of the M. leprae β subunit gene of RNA polymerase. This position was not a so-called “hot spot” of rpoB-associated resistant mutations (8); however, it induced a change of amino acid residue from aspartic acid to glycine (not shown). There was no relationship among the resistant samples, and no double mutation encoding both folP and rpoB genes was observed.

It is not easy to determine whether the resistant strain developed before or after introduction of MDT. However, there might be some patients who are inadequately treated with MDT due to economical or other social reasons. These patients have a higher risk to produce multidrug-resistant strain than patients adequately treated. Active surveillance is required for control of the spread of drug resistant M. leprae.

Taken together, we showed that some leprosy patient contacts have been infected with M. leprae. Also, dapsone resistance has been detected in Pakistan.

Acknowledgment. We thank Dr. Akira Kobayashi and Dr. Tetsu Nakamura (Peshawar-Kai Hospital, Peshawar, Pakistan) for supplying clinical samples. This work was supported in part by a Health Science Research Grants-Research on Emerging and Re-emerging Infectious Diseases, Ministry of Health, Labour and Welfare, Japan.

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REFERENCES
Phimosis as a Presenting Manifestation of Genital Involvement in Leprosy

TO THE EDITOR:

Though testicular involvement is well known in leprosy, there are only few published reports on the presence of lesions of leprosy on male genital skin. In most of the cases reported, lesions were present on the scrotum with or without involvement of penile shaft, prepuce or glans. We report herein, a case of borderline lepromatous (BL) leprosy in type 1 reaction with cutaneous lesion over the prepuce presenting as phimosis.

CASE REPORT

A 20-year-old male patient presented to us with complaints of erythematous painful swelling of the prepuce with inability to retract for the duration of 1 week. Patient had noticed asymptomatic hypopigmented patch over the same site since 6 to 8 months for which he did not take any treatment. Apart from this, he was not aware of any lesion over other parts of the body prior to this episode. On examination, there was an erythematous, tender, plaque present circumferentially over the prepuce resulting in inability to retract prepuce (The Figure). Further examination revealed multiple (25 to 30) erythematous hypoaesthetic plaques with loss of loss of hair and minimal scaling, measuring from 1 to 5 cm in size over trunk, limbs and one small lesion over the scrotum. His right ulnar nerve, greater auricular nerve, and left common peroneal nerves were thickened with no sensory or motor deficit in the area of their distribution. There was mild tenderness involving right ulnar nerve alone. On slit skin smear examination from ear lobes and lesions (4 sites), BI was 1+ and skin biopsy from one of the lesions showed histopathological features consistent with BL Hansen. Patient was started on World Health Organization (WHO) multi-drug therapy (MDT) multibacillary (MB) regimen and Tab. prednisolone 30 mg daily. Within a week of starting treatment, swelling and tenderness regressed almost completely and there was no difficulty in retraction of prepuce. Prednisolone was gradually tapered over the next 12 weeks and patient has continued to do well at six months follow-up.

DISCUSSION

Although no part of the skin is immune from invasion by Mycobacterium leprae (1), the genital skin has been described as an unusual site for leprosy (1). Genital skin has been reported to be relatively cooler than the core temperature under experimental conditions, and thus expected to be at increased risk of infiltration by Mycobacterium leprae (1). However, due to the use of occlusive undergarments, it is likely that the temperature of the genital skin may not remain that low and this elevated temperature may possibly make this area less prone to the development of leprosy lesions (1).

Clinical involvement of the genital skin in leprosy has not been studied widely, largely because of the inability in examining patients in totality in routine clinical set-up. Fox and Knott (5) first time reported involvement of male genitals in the form of leprous nodules on the scrotum, prepuce and glans. Parikh, et al. (6) reported six cases of borderline leprosy with lesions on scrotum and penis. Dixit, et al. (7) reported presence of scrotal lesions in tuberculoid leprosy. Kumar, et al. (8) observed genital lesions in 6.6% of all male cases of leprosy. They were seen most frequently in lepromat-
tous leprosy (25.8%) followed by borderline lepromatous (13.3%) and borderline tuberculoid (1.4%) leprosy. Arora, et al. (1) found genital lesions in 2.9% of the cases with borderline disease. Most of their patients belonged to the borderline group and were in type 1 reaction. Rarely, histoid lesions have also been reported on the male genitals (7, 10).

Mycobacterium leprae has been found in the dartos muscle of scrotum even after adequate therapy (11). Pandya and Anita (8) have reported leprous granulomas and AFB in one third of biopsies from the scrotal skin in patients with all types of leprosy even in the absence of lesions on scrotum. Our case presenting with leprosy lesion on prepuce as phimosis is probably first of its kind. Recently published reports (6, 12) indicate that genital skin lesions in male leprosy patients are not as uncommon as suggested previously. These lesions are missed either because they are not looked for carefully or reluctance on the part of patients to expose the genitals. Therefore, genital examination of leprosy patients is important not only to document its involvement but also to find out any other associated disease, which will require more attention than mere documentation.

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5. KAUR, S., and KUMAR, B. Study of apparently uninvolved skin in leprosy as regards bacillary population at various sites. Lepr. Ind. 50 (1978) 38–44.
Lepromatous Leprosy with Extensive Unusual Ulcerations and Cachexia. Is It the First Case of Lucio’s Phenomenon from Iran?1

ABSTRACT
We report a 33-year-old Iranian woman with widespread ulcerative lesions in the setting of lepromatous leprosy. We think that the sudden appearance of the characteristic necrotic lesions in the absence of fever and other systemic manifestation, and in accordance with epidermal necrosis and the presence of large numbers of AFB in the endothelium are all in favor of the diagnosis of Lucio’s phenomenon for this patient. To our knowledge this is the first patient who may have had this phenomenon reported from the Middle East.

RÉSUMÉ
Nous rapportons ici le cas d’une femme iranienne de 33 ans qui a présenté des lésions disséminées d’ulcérations cutanées dans le contexte d’une lèpre lépromateuse. Nous pensons que la soudaine apparition de lésions nécrotiques caractéristiques, en l’absence de fièvre et d’autres manifestations systémiques, et en tenant compte de la nécrose épidermique et de la présence de très nombreux bacilles AAR dans l’endothélium, est en faveur du diagnostic de phénomène de Lucio pour cette patiente. A notre connaissance, il s’agit du premier cas rapporté au Moyen-Orient qui a probablement été victime de ce phénomène.

RESUMEN
Presentamos el caso de una mujer iraní de 33 años con lesiones ulcerativas diseminadas asociadas a lepra lepromatosa. Pensamos que la aparición súbita de las lesiones necróticas características, la ausencia de fiebre y otras manifestaciones sistémicas, la necrosis epidérmica y la presencia de grandes números de bacilos ácido-resistentes, indican un caso de fenómeno de Lucio en esta paciente. Hasta donde sabemos, este es el primer caso de un paciente con fenómeno de Lucio en el Medio Este.

TO THE EDITOR:

Northern Iran was an endemic area for leprosy until a few years ago and almost all of the clinical variants of leprosy have been seen in this area. We report here a case of lepromatous leprosy with unusual extensive ulcerations that we think might be the first case of Lucio’s phenomenon from the Middle East.

A 33-year-old woman with a long history of skin lesions was referred to our service after her lesions had started to become generalized and necrotic, a few days previous. She was inhabitant of a far located rural area. Her father was a known case of lepromatous leprosy and had been treated with dapsone as monotherapy during 1970’s and later with standard multiple drug therapy (MDT) for leprosy, but the patient had never visited a dermatologist nor received any antileprosy medications.

On admission to hospital, the patient was cachectic and in poor general condition. An obvious leonine face with nearly complete loss of eyebrows and eyelashes, and extensive ecchymotic patches in association with deep ulcerative lesions were seen on her body, especially on the extremities (Figs. 1 and 2). On laboratory examinations, a full blood count showed anemia with Hb 4.8 g/dl. The white cell count was normal, but platelet count was raised to $7 \times 10^5$/dl. The erythrocyte sedimentation rate was 72 mm/hr. Rheumatic factor and C-Reactive Protein were weekly positive but other laboratory tests including, renal function tests, liver enzymes, electrolytes, and coagulation tests were all in the normal range.

Slit skin smears from different locations showed multiple acid-fast bacilli (AFB),
and a skin biopsy from an ecchymotic patch revealed swollen endothelial cells with fibrinoid necrosis and a mixed inflammatory infiltrate with nuclear dusts and an atrophic epidermis with focal necrosis. In the dermis, enormous numbers of AFB were seen with Ziehl-Neelsen staining. AFB were also present in the form of clumps in the vessel walls. We started supportive care and a standard antileprosy therapy including dapsone 100 mg daily, clofazimine 50 mg daily and rifampicin 600 mg monthly for the patient. A combination of systemic antibiotics including a 3rd generation cephalosporin and vancomycin was also started after the patient became febrile. During the admission, the patient’s hemoglobin dropped and she developed hematuria and pancytopenia and unfortunately died at the 35th day of admission because of severe sepsis.

Lucio’s phenomenon is a severe necrotizing reaction occurring in the diffuse, lepromatous leprosy. Lucio and Alvarado first described this phenomenon in 1852. Latapi and
Chevez later denoted this reaction in 1948 as Lucio’s phenomenon (3). This type of reaction is most commonly seen in Mexico and Central America (3, 6, 8), and is rare outside America, although Saoji, et al. reported two cases of this phenomenon from India (9), and Ang, et al. recently reported two Chinese men with fatal Lucio’s phenomenon (1). Painful macules or plaques progressing to ulcers characterize Lucio’s phenomenon. Features of the underlying lepromatous leprosy commonly described include diffuse thickening of facial skin, maderosis, and destructive rhinitis (4). Anemia, lymphadenopathy, splenomegaly, hypoalbuminemia, hypocalcemia and polyclonal gammopathy are among the other reported manifestations (4, 6).

Rea, et al. have hypothesized that patients with Lucio’s phenomenon have an exceptionally deficient defense mechanism, allowing unrestricted proliferation of AFB in endothelial cells, facilitating contact between bacterial antigens and circulating antibody and leading to infarction. Also, this nadir of resistance allows unimpeded dissemination of AFB, accounting for the clinical features of diffuse non-nodular leprosy (7). Most cases of Lucio’s phenomenon have been reported to have a leukocytoclastic vasculitis as the underlying pathologic abnormality, although some cases of Lucio’s phenomenon may be caused by vascular damage due to direct invasion of *Mycobacterium leprae* and not necessarily by leukocytoclastic vasculitis (4). Deposits of mixed-type cryoglobulins...
(IgG, IgA, IgM, C3, and C1q) have been observed in dermis vessels affected by vasculitis of the Lucio’s phenomenon type, suggesting a mechanism mediated by deposits of immune complexes (5).

Lucio’s phenomenon occurs in patients with undiagnosed and untreated leprosy, whereas the erythema nodosum leprosum (ENL) may occur in any type of lepromatous leprosy, and frequently occurs after starting the treatment. The main clinical differences between the Lucio’s phenomenon and ENL are that the former is an ulcerative reaction occurring in the absence of cutaneous nodules, whereas the latter usually present as tender cutaneous nodules that rarely ulcerate (1). Absence of fever, leukocytosis, tenderness, and other systemic presentations such as arthritis, neuritis, and iridocyclitis, and failure to respond to thalidomide are among the other distinct features of Lucio’s phenomenon (6, 10). Histologically, Lucio’s reaction can be distinguished from ENL by epidermal necrosis and by necrotizing vasculitis manifesting necrosis in the walls of superficial vessels and severe, focal endothelial proliferation of mid-dermal vessels. Furthermore, the numbers of AFB are much more in the lesions of Lucio’s phenomenon in comparison with The ENL (7). In addition to ENL, in the differential diagnosis of Lucio’s phenomenon, vasculitis of other origins should be considered, such as the antiphospholipid antibody thrombotic syndrome associated with lepromatous leprosy (5).

Although we were not able to assay cryoglobulins, cryofibrinogens, and antiphospholipid antibodies in this patient, normal coagulation tests including partial thromboplastin time make this syndrome improbable for this case.

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REFERENCES

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

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<td>4–7</td>
<td>Mar-04</td>
<td>Cancun</td>
<td>11th International Congress on Infectious Diseases</td>
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<td>15–17</td>
<td>Apr-04</td>
<td>Quebec City</td>
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<td>19–22</td>
<td>May-04</td>
<td>Beijing</td>
<td>IX International Congress of Dermatology</td>
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<td>18–23</td>
<td>Jul-04</td>
<td>Montreal</td>
<td>12th International Congress of Immunology</td>
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<td>7–11</td>
<td>Nov-04</td>
<td>Miami</td>
<td>53rd Annual Meeting of the American Society of Tropical Medicine &amp; Hygiene</td>
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<td>Nov-04</td>
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<td>Bangkok</td>
<td>9th Western Pacific Congress on Chemotherapy and Infectious Diseases</td>
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Notice. The following announcement was received from the Pan American Health and Education Foundation.

Call for Nominations. 2004 Fred L. Soper Award for Excellence in Health Literature. The Pan American Health and Education Foundation, a non-profit U.S. based public foundation, collaborating partner of the Pan American Health Organization (PAHO) is accepting nominations of outstanding scientific journal articles of public health in the Region of the Americas that were published in 2003.

To be nominated, an article must have been published in a scientific journal that is listed in the Index Medicus (MEDLINE). Authors must have a principle affiliation with a teaching, research or service institution located in the Region of the Americas. Preference is given to studies involving more than one discipline and to papers related to infectious disease.

Eligible papers may consist of a report, an analysis of new data (experimental or observational), or a new approach to analyzing available data. The award consists of a certificate of merit and a cash prize of U.S.$2,500.

Nominations must be received not later than 30 June 2004.

Address for sending nominations: Fred L. Soper Award Committee, Pan American Health and Education Foundation, 525 Twenty-third Street, N.W. Washington, D.C. 20037. Phone: 202-974-3416. E-mail: foundation@paho.org

The following grants were announced in the New Awards section of the October 2003 issue of TDR News.

- Gareth Wyn Griffiths, European Molecular Biology Laboratory, Heidelberg, Germany. Analysis and manipulation of mycobacterial phagosome signalling networks. (Budget: U.S.$35,000)
- Stewart Thomas Cole, Institut Pasteur, Unite Genetique Moleculaire Bacterienne, France. Post-genomic leprosy diagnostics. (Budget: U.S.$33,500)
- Mariane Martins de Araujo Stefani,
Brazilian Hansen’s Disease Annual Meeting was held in Brasília from 3–4 November, sponsored by the Brazilian Ministry of Health (MoH). Participants were from Control Programs at National and State level, Pan-American Health Organization, World Health Organization (WHO), National Hansen’s Disease Committee, Non-Governmental Organization—ILEP, MORHAN—Movimento de Reintegração de Pacientes de Hanseníase, Directors of National Reference Centers and Research Institutes. We are presenting a short report on the meeting and its main recommendations in consonance with the Executive Summary.

1. In opening session, Dr Jarbas Barbosa da Silva Junior, Chief Director of “National Surveillance Secretariat” branch, reinforced the political commitment of the Brazilian government towards Hansen’s Disease elimination. He also stated the relevance of Hansen’s Disease Annual Meeting for evaluating the elimination progress and to set priorities for control and research activities in the context of the infectious disease.

2. *Current Hansen’s Disease epidemiological situation:* Brazil is the most endemic country in Latin American congregation almost 90% of the newly detected cases (NDC). Hansen’s Disease epidemiological situation is still considered endemic at State level with a large variation in prevalence and detection rates among regions. In 2002, the point prevalence was 4.4 per 10,000 inhabitants and more than forty thousand of new cases were registered (2.40 per 10,000 inhab). In the last decade, trends in prevalence declined sharp but detection rates remained stable in the same period. Although, there is an effort to detect cases as early as possible around 6% of the NDC still have grade II disability. Nearly 10,000 annual Hansen’s Disease hidden cases were estimated which could underestimate the prevalence figures. Since 1996, the Brazilian Hansen’s information system is on-line, http://hanseniae.datasus.gov.br/hans/hans.htm, providing data by municipality for epidemiological and management purposes.

3. *World Hansen’s Disease Rank:* For the first year, the WHO ranked Brazil as the top Hansen’s Disease country according to the prevalence rate. However, the use of the prevalence as the solely indicator of Hansen’s Disease elimination has raised several methodological issues and controversies. Little attention has been given to the differences in the inclusion and exclusion criteria of patients in the Hansen’s Disease register making the prevalence rates among countries incomparable. For example, in Brazil the prevalence rate would fall by half (2.1 per 10,000 inhab.) if calculated according to WHO parameters. This meeting recommended incorporating NCD rates and NCD rates among children as additional indicators to monitor Hansen’s Disease elimination in Brazil.

4. *Current operational aspects:* There was an effort to decentralize the Hansen’s Disease control program by integrating its activities practice and in the primary care (family doctors) in the general health services. Within all health system, we acknowledge that there are many skilled health workers engaged in Hansen’s Disease control activities at all levels. However, poorly structured health system/inadequately funded makes this integration process slow and uneven throughout the country.

5. *Research recommendations:* Two main recommendations were made: (i) to build-up a research network to strengthen operational studies and also to bridge basic research with field priorities; (ii) the Brazilian independent U-MDT proposal was presented/discussed and considered a research priority by MoH. Another research proposal MDT-A was discussed and the MoH restated its previous position to use non-supervised regimens, as an exception, only in remote areas of the country.

Finally, the Brazilian Ministry of Health has an official Scientific Committee to support its policies and recommendations. The current members are: Joseney Raimundo Pires dos Santos—MoH; Gerson Fernando Mendes Pereira—MoH; Euzenir Nunes.
Un nouvel atlas de la lèpre—the French version of “A new atlas of leprosy”

A new atlas of leprosy, by Drs. A. C. McDougall and Yo Yuasa, has been demonstrated to be a very useful material for teaching and self-learning of leprosy among health workers in the field. Now the Atlas has been translated into French by Dr. Pierre Bobin, and sponsored jointly for publication by the Sasakawa Memorial Health Foundation and the Association Française Raoul Follereau.

Copies of the French version Atlas can be ordered, free of charge, from the Association Française Raoul Follereau by post (Madame Bénédicte de Charette, Association Française Raoul Follereau, 31 rue de Dantzig, 75722 Paris Cedex 15, France) or by e-mail (direction-aide@raoul-follereau.org).

ILA Global Project on the History of Leprosy

The ILA Global Project on the History of Leprosy convened three sessions at the 6th European Association for the History of Medicine and Health Conference in Oslo, 3–7 September 2003. The theme for the conference was “Health Between the Private and the Public—Shifting Approaches.”

The first panel “Mapping the Geography of Leprosy: the Politics of Bodies and Boundaries” consisted of six papers which traced the connections between bodies determined by disease and spaces determined by policy. The histories and the medical geographies of leprosy in the United States, Canada, Colombia, South Africa, and Eastern Nigeria indicated that while late nineteenth century and early twentieth century policies of exclusion, isolation, and segregation impacted on individual bodies, the same policies were instrumental in delineating society and the nation.

The second panel presented papers from Brazil. This panel considered leprosy as a public health issue. Initially it focussed on the proposals of the “Comissão de Profilaxia da Lepra” (conducted in 1915 and 1919) and the Inspetoria de Profilaxia da Lepra e Doenças Venéreas (established in 1920); then it described the influential policies adopted against leprosy in 1930 in the State of São Paulo that resulted in an authoritarian and arbitrary prophylactic model of fighting leprosy. Finally, the life of Adolpho Lutz and the controversies regarding leprosy transmission in Brazil were examined.

Then the panel on leprosy in India located the shifts and changes associated with leprosy over the 1850–2001 period viewing the disease from colonial medical, social and oral history perspectives. The first paper examined the nuances of colonial enumeration in western India. The subsequent papers focussed on the leprosy patient: firstly, in the period from 1900 to 1955 in Orissa, in eastern India and then from the point of view of oral evidence of leprosy patients.

The project also held the inaugural meeting of the history of leprosy academic network. Future strategies for the network include establishing an electronic discussion forum, encouraging the submission of papers to history of medicine conferences, and developing possible collaborations amongst members of the network as a result of the intention of the Wellcome Unit for the History of Medicine at Oxford to make the history of leprosy one of its priority research areas in its latest bid for funding from the Wellcome Trust.

ABSTRACTS

Leprosy and its Meanings: the Body and Society. Jo Robertson

This paper considers the consequences of leprosy’s accumulated representational his-
tory as its meanings circulate endlessly between individual bodies and within the complexities of the medical and the social spheres. The idea of the disease triggers off a whole range of extremely complex associations: not only is leprosy abhorred, it is also sentimentalised, exoticised, romanticised, and orientalised. In addition, leprosy is appropriated in different ways, often for political purposes, over time, so that the representational force of leprosy shifts, merges with other diseases and discourses, and seems to vanish, but inevitably re-emerges reinforced with revivified symbolic resonance. In order to explore some of the reasons for the obdurate and unyielding imaginings attached to the disease, bearing in mind that these have very real consequences for people with leprosy, this paper draws upon Mary Douglas’s argument that leprosy is used to contain and express social disruption and reorganisation. Trying to contain the representation of leprosy, that is trying to control the way it is used, is therefore, if not a futile, a never-ending task.

The Leprosy Patient, Society and History: Orissa 1900–1950.
Chandi P. Nanda and Biswamoy Pati

This paper begins by examining popular, adivasi (viz. tribal) perceptions of leprosy and the initiatives undertaken by the ruling chiefs of Keonjhar, including the structure of legitimacy and incorporation/cooption of adivasis by the princely state of Keonjhar. It then describes how the colonial health establishment located leprosy within a concern for public health, and delineates the ambiguities and inner conflicts related to leprosy interventions in Orissa within the colonial establishment. These conflicts resulted in a low priority being given to the disease, and demonstrate how, in many ways, colonial intervention reinforced the inherited perception of leprosy as god’s curse. Finally, the paper examines the life of the people inside the leprosy asylum at Cuttack, (established in 1919), and unravels some of the complexities involved in the way they negotiated it. These negotiations were influenced by shifts and changes linked to discoveries related to the cure of leprosy in the 1920’s, and were also reinforced by Gandhian efforts to work for the dignity of those affected by the disease. While these developments opened up new possibilities, they nevertheless did not do away with the problem of “confinement.” Viewed from the point of view of those affected by the disease, this suggests a continuity, that seems to be visible in post-colonial Orissa. In fact, as emphasised, the report of an inmate’s suicide in 1953 demonstrates the agonised existence and alienation suffered inside the asylum.

Nineteenth Century Indian Leper Censuses and the Doctors.
Shubhada S. Pandya

[The word “leper” is used solely because of its historical accuracy; there is no intention to disparage people with leprosy].

This paper focuses on two instances of medical analysis of leprosy census data in 19th century colonial India: the first, the Bombay Presidency leper census of 1867 by Henry Vandyke Carter; the second, the national decennial censuses of 1871 to 1891 (in which lepers were also enumerated) by the Leprosy Commission, which visited India 1890 to 1891. The paper questions the relationship between the medical preconceptions about leprosy and their conclusions based on the analyses. It has been suggested that periodic counting, classification and categorization of the Indian population enabled British colonialism to “know” and control, its subjects. In this instance, enumeration of lepers was proposed by the authorities as a prerequisite for disease containment.

The Bombay Presidency Leper Returns of 1867 listed the name, age, sex, caste and place of residence of over 10,000 lepers, and also indicated whether or not a leper had a similarly afflicted relative. All castes were found to be prone to leprosy. Henry Vandyke Carter was already a supporter of the hereditary theory of causation when he set about his analysis. He invested the notion of the hereditary transmission with power over every character of leprosy revealed in the census returns, from widely varied regional and sub-regional prevalence rates, to variable sex ratios, to the rare instances where a great grand-parent was listed as a leper. He was undaunted by the inconvenient fact that over 80% of the listed lepers had denied a family history, attributing it to a reluctance on their part to admit the “truth.” Like his colonial contem-
poraries who viewed Indians through the ethno-sociological prism of caste, Carter made the Indian leper “comprehensible” through his fixed place in the caste map and the customary practice of caste endogamy. In this scenario, the leper’s body, burdened with the leprous seed from pre-history, inexorably shackled by caste exclusivity and caste endogamy, could never free itself of the hereditary “taint.” Indian leprosy became a paradigm of biological determinism.

Bureaucrats supervising the decennial imperial census operations in the later decades of the 19th century were aware from the outset that the leper statistics that had been generated were, for various reasons, too flawed for definite conclusions to be drawn. The strongest member of the Leprosy Commission that visited India during the panic and lepraphobia engulfing Europe in the Damien aftermath, was Beaven Rake. He was exceptional in being an avowed skeptic of the contagion theory, even in the case of Damien. The Commission felt obliged to respond to alarmists who alleged that uncontrolled Indian leprosy posed an “Imperial Danger.” In order to achieve their objective, the members showed fewer reservations than the bureaucrats about using questionable census statistics. They calculated leprosy trends in selected districts using anomalous figures, and demonstrated to their satisfaction that Indian leprosy was not increasing, and if anything, decreasing.

Thus in both instances, analysis of census data provided an avenue to embellish and legitimate the preconceived notions of the physicians about the nature of leprosy in India. Despite the official claim that leper censuses were necessary for disease control, the data generated never resulted in systematic measures towards this end in India.

The intervention of MDT from the 1980’s, with a very concrete message that leprosy is easily curable heralded a new era for leprosy control, cure and rehabilitation. The decline in prevalence and the large numbers of visibly cured patients combine to create a wholly different environment. With a view to examining social rehabilitation, we ask to what extent these interventions have made a dent on public prejudice (urban and rural) against leprosy; whether prejudice continues to be directed against cured patients, (both with and without physical deformities); and we examine the situation of patients who are undergoing treatment, and of their families.

The paper looks at one Indian approach to social rehabilitation, the unique philosophy of the “Art of Living Foundation,” which offers programs with yoga breathing and simple meditation to remove stress (“breathe out stress”), enhance self esteem, and increase self confidence and self reliance. Similar programs have already been offered in India and overseas for the visually challenged, for those who are socially deprived, for those involved with substance abuse and for prison inmates. (Information on this non-profit foundation, which works with ECO-SOC of the United Nations, may be found on their website http://artofliving.org ). Oral History is used to document this leprosy and meditation project of the Art of Living Foundation, and, in the process, the uses of oral history are critically examined.

**Meditation and Social Rehabilitation of Leprosy Affected? An Oral History Study of this Unique Project in Contemporary India. Sanjiv Kakar**

The paper looks at the developments in leprosy intervention strategies in India during the last two decades, specifically at the success in leprosy control. This is substantiated by the decline in prevalence from 51 cases per 10,000 in 1981 to 4.2 per 10,000 in March 2002.

The State, Physicians and Leprosy in Modern Colombia. Diana Obregón

In the early twentieth century, leprosy became an obstacle for the civilizing and modernizing project of the Colombian elites. The Colombian government, with the expert assistance of the medical community, started to take control of lazarettos, and physicians began to medicalise leprosy. The government enacted extremely severe laws in order to control lazarettos. Their main purpose was to block the social and economic links between the town-lazarettos and the external world. The rationale for this was to arrest the spread of the disease. The government also attempted to expel from the lazarettos a large population free of leprosy, mainly composed of relatives of leprosy sufferers, who were confined within
leprosaria. The period in which the Colombian State began to control leprosaria coincided with the formation/modernization of the Colombian State. Refinement of the arts of government, definition of citizenry (for example, through the establishment of such obligations as denouncing victims of leprosy), and exclusion of a social group defined as ‘lepers’ came together. A disease-apart approach was institutionalized by establishing two distinct domains of public health: a special official agency was set up for leprosy, while all other diseases were handled through a different department. However, in spite of the efforts of physicians and the government, leprosy was not thoroughly medicalized. Patients actively opposed compulsory segregation with attempts at converting lazarettos into prison-asylums. Non-leprosy sufferers remained at the lazarettos, and scientific medicine competed with popular healers, herbalists, and charlatans within these institutions. After all, these had been ordinary towns until the state took control of them in the early twentieth century. Since leprosaria were not hospitals, physicians were unable to order treatments. The medicalization of leprosy was only partially accomplished because of its demarcation as a disease-apart.

The Spaces of Exclusion in American Public Health: The Case of Leprosy.
D. George Joseph

“Only when history and geography are integrated,” wrote Erwin Ackerknecht, “will they reveal a genuinely true picture of conditions are they are” (Geschichte und Geographie der Wichtigsten Krankheiten, 1963, p. 2). Historians of medicine, however, have largely neglected geography in their social studies of disease and public health, and only recently, have spaces and places emerged as a discursive and analytical category among historians. The anthropologist Mary Douglas has described the human experience with leprosy in terms of the associations of moral contamination, defilement of the physical body, and pollution of the environment. In bringing together Ackerknecht’s idea of place in historical understanding with Douglas’ ideas about the stigma of leprosy, it becomes clear that discussions of geography and space-real and perceived, whether the physical space of the sufferer’s body or the physical grounds of the leprosarium or the physical and social exclusion from the community sufferers endure—must enter into any complete examination of leprosy’s history.

This paper considers American efforts to control leprosy in the late nineteenth and early twentieth centuries in three different geographic and socio-political contexts: (i) Penikese Island, Massachusetts, reflecting the attempt in one locality to segregate patients before the creation of a national program of leprosy control; (ii) Carville, Louisiana, emblematic of a federal program of leprosy quarantine; and (iii) Molokai, Hawaii, reflecting acts of exclusion and segregation within a wider imperial agenda. A comparison of the three sites reveal that the leprosaria were spaces in which its residents were subject to exclusion, classification, and inquisition and that there was a fluid exchanges of ideas and practices between the geographic and socio-political contexts. The leprosaria also served as “moral architecture” as these attempts at leprosy control involved the physical and social re-ordering of bodies and geographic spaces to achieve their social and medical goals of eliminating the dangers of contagion and delineating the boundaries and the distance between the sick and healthy, the dangerous and safe, and the pure and impure.

Isolation and social exclusion within a segregated society: a case study of Westfort Leper Institution, South Africa, 1898–1948. Simonne Horwitz

Seven miles west of Pretoria, South Africa’s administrative capital, on a tract of 1200 acres of semi-arid land, lies the remains of the Westfort Leper Institution. The new millennium saw plans being put in place to convert the shell of the leprosarium into a top tourist attraction with a casino, lavish hotel, and an adjoining community housing project. This use of the site, as a vibrant and inclusive social center, is in sharp contrast to a history that was marked by isolation, segregation, and social exclusion. This paper argues that, during the first half of the twentieth century, segregation and exclusion at Westfort Leper Institution operated on multiple levels. Not only were patients isolated from the outside world, but they were internally segregated according
to gender, class, and race. Through detailed case studies, this paper demonstrates how these factors influenced the daily lives of those living with the disease, the way in which they were managed, and importantly, their access to facilities and resources. These divisions were highlighted by the fact that Westfort catered for male and female patients and was one of only two multiracial government-run leprosaria in South Africa. Unlike the leprosarium on Robben Island, Westfort was planned and built under a Boer and not a colonial government that, as this paper suggests, lead to a very different political and historical trajectory. With the closure of the leprosy asylum on Robben Island in 1931, Westfort became the only remaining multiracial leprosy asylum during the critical period when South Africa was becoming an increasingly racially segregated state. An examination of the institution during this period shows that segregation and social exclusion were increasingly seen not only in the way patients were treated and in the leprosy legislation, but in the physical construction and reconstruction of the institution, hence reinforcing the focus on segregation as opposed to curative approaches to the disease.

John Manton

This paper attempts to reproduce, in outline, a discursive space in which ambiguities relating to leprosy and its control, within the northern and eastern sections of colonial Ogoja Province, Nigeria, can be fielded. Leprosy control in this area was administered on behalf of the Nigerian government by the Roman Catholic Mission (RCM), through a series of central settlements and satellite segregation villages, the first of which were founded in 1945. It is difficult to convincingly reconstruct either a history or a geography of leprosy prevalence in Ogoja Province, an area largely marginal to British colonial concerns, whose demographic and linguistic patterns were poorly understood by administrators and missionaries alike. One of the main purposes of this paper, then, is to outline a cognitive map of Ogoja Province according to which the confusing diversity of statements about leprosy can be interpreted as constituting and reflecting a political arena encompassing the desires and needs of leprosy sufferers, African communities, Catholic missionaries and British colonial administrators.

How were colonial and missionary misapprehensions of Ogoja politics and society manifested in the evolution of leprosy control? The contention surrounding issues of taxation, wages, markets and charging for medical services, and the extent to which these issues, incompletely distinguished one from the other, emerged as facets of essentially the same administrative problem, provides one of the clearest indications of the epistemological crises at the core of colonial medicine and administration in Ogoja. I examine the social construction of destitution with regard to colonial expectations of customary family duty, the issue of payment for leprosy services and the evolution of competing and accompanying RCM-run “clean” dispensary services, and the recurrent theme of leprosy-patient access to local market facilities.

This paper exemplifies a broader concern with role of the RCM, and of leprosy control, in the making of political forms for the administration of Ogoja in the context of the post–1945 formation of development ideologies, policies and agencies. As with much in the administration of colonial West Africa, these policies were characterised by a surprising lack of control. The porosity of leprosaria, as physical, conceptual and organisational spaces mitigated the force of compulsion, the success of case-finding and the development of research, acting to problematise the relation of leprosy control to its outcomes.

Contagion, Containment, and Exclusion: The Spatial Governance of Leprosy and Chinese Immigration in British Columbia.
Renisa Mawani

This paper explores the spatial and racial dimensions of leprosy management in late nineteenth and early twentieth century British Columbia (BC). In 1891, Victoria’s Medical Health Officer detected the first cases of leprosy when five Chinese men believed to be afflicted with the disease were found in the City’s Chinese quarters. In response to pervasive and growing local fears about “Chinese leprosy,” the City responded
quickly by creating a containment facility on D’Arcy Island, a small island located seventeen miles north-east of Victoria. Operative between 1891 and 1924, the island became a quarantine and detention facility that housed a total of 49 men, 43 of whom were Chinese. Although the lazaretto was intended to be a therapeutic and corrective space, island residents were offered virtually no treatment and were sent to the island to be spatially contained and in some cases, exiled from the nation through deportation orders. Through a detailed examination of government correspondence, newspaper coverage, legislation, and other archival sources, I explore the management of leprosy on D’Arcy Island and consider the ways in which anxieties about hygiene, sanitation, and public health were tied up with ongoing concerns about Chinese immigration and the protection of national borders. Although government officials—at the local, provincial, and federal levels—responded to escalating anxieties about leprosy through a number of repressive and coercive strategies (ranging from quarantine detention, to immigration restrictions, and in some cases deportation orders), I argue that the management of “Chinese leprosy” was always geographically configured. Quarantine, segregation, and deportation were all legally mandated spatial techniques that set the physical and discursive parameters of national in/exclusion. But while the care and control of leprosy was endeavored through particular geographies of exile, I suggest that D’Arcy Island must be contextualized within the broader climate of Chinese exclusion that shaped BC at that historical moment. Thus, the spatial management of leprosy was only one dimension of a wider political agenda aimed at constructing a provincial and national identity, by physically separating Chinese from European, diseased from healthy, and foreigner from citizen.

The Spatial Politics of an African Leprosarium. Harriet Deacon

Historically, the treatment of people identified as having leprosy has been heavily reliant on practices of exclusion from society on the basis of gender, race and class segregation within institutions. In colonial South Africa, people with leprosy were stereotyped as sexually deviant and black and until the 1890’s only the destitute entered leprosaria. This paper provides a geographically-informed analysis of the Robben Island leprosarium, established in 1846 and closed in 1931. It was an early example of a state-run leprosarium under medical supervision that housed under a hundred destitute, mainly black, voluntary patients, until compulsory segregation in the 1890’s brought in five or six times that number, and more white patients. The paper argues that gender segregation had a greater impact on the Robben Island institution than any other form of patient segregation. All female leprosy patients were removed from Robben Island in 1871. This was not only because doctors and officials needed to show that they were preventing hereditary transmission of the disease, but because they could not use force to prevent contact between the sexes on the island without appearing illiberal. In the 1880’s, when the female leprosy patients were returned to the island, heredity was still accepted as a possible mode of transmission in spite of the discovery of the leprabacillus. Fear of the transmission of the disease to whites (by heredity as much as by contagion), and especially through sexual contact, was more important in justifying stricter segregation laws in the 1880’s and 1890’s than theories of contagion alone. Theories of contagion changed over time, too. In the early 1890’s, the key path of infection was thought to be the physical bodies of leprosy patients: once the leprosy wards were empty, they could be reused. By the 1930’s, however, the environment of the leprosy patient was also considered tainted. Empty wards had to be totally destroyed by fire and demolition.

Leprosy as a public health issue: the Comissão de Profilaxia da Lepra (1915–1919) [Brazil]. Laurinda Rosa Maciel

This paper investigates activities and discussions developed by the members of the “Comissão de Profilaxia da Lepra,” between 1915 and 1919, that led to proposals concerning leprosy prevention and control. Subcommittees were established by the Commission with existing social and political concerns in mind. Leprosy and occupations, leprosy and housing, leprosy and iso-
lation, leprosy and marriage, leprosy and immigration and leprosy transmissibility were identified as topics for investigation. These sub-committees were composed of scientists from different institutions who, in this climate, were able to exchange ideas and theories. The main conclusion derived from the work of the Commission was that it would be impossible to continue to take care of leprosy without the engagement of an official institution, related to the Ministério da Justiça e Negócios Interiores. After the final discussions, the Commission presented several suggestions to the Government concerning leprosy care. One of the most relevant was the need to create an office for managing public health and leprosy control in Brazil, considering that the disease was probably a public health issue.

In 1920, the Inspetoria de Profilaxia da Lepra e Doenças Venéreas was created, establishing a link between public health, leprosy, and venereal diseases. This study reveals diversified aspects concerning the creation of the first office for disease control, emphasizing the problem of leprosy in Brazil. This text is part of a larger research project dedicated to the history of leprosy in Brazil between 1920 and 1962.


At the beginning of the twentieth century, the eradication of epidemics and the control over endemic diseases were regarded as important issues for economic development in Brazil. At that time, however, leprosy represented a serious problem to be solved. For example, in 1930, the State of São Paulo, the most developed and richest of the country, adopted policies to fight leprosy, thereby influencing the entire nation. The prophylaxis policy of São Paulo was grounded on: (i) “The Norwegian Model,” which recommended the isolation of the leprous; (ii) eugenics ideals, which proposed the exclusion of the “undesirables”; and (iii) “The Campaign Model,” which was inspired by military organization. Based on those concepts, prophylaxis started to be considered as a battle, and the disease, as the enemy to be exterminated. Each and every sanitary measure represented a struggle, the consequences of which resulted in the sacrifice of individual rights to the wider demands of the community. Subsequently, the compulsory isolation of all the leprous was enacted, without any consideration of the manifestation or stage of the disease, or gender or age, and the Department of Prophylaxis of Leprosy was empowered with a strict, hierarchical and centralized organization. The prophylaxis measures continued in São Paulo until 1967 against international recommendations and even against the Brazilian legislation, which had already abolished isolation in 1962.

This study is based on the legislation on leprosy, specialized literature, minutes of medical conferences, protocols from the prophylaxis service and medical records. The aim of this paper is to analyse how the State, in the name of preserving public health, was able to intervene in every aspect of the life of the sick, to the extent of interfering with their families, affecting healthy parents, partners and children. Our objective is, as well, to discuss how the authoritarian and arbitrary prophylactic model of fighting leprosy, adopted in São Paulo, was socially and politically welcomed in the country.

**Adolpho Lutz and the Controversies Regarding Leprosy Transmission in Brazil. Jaime Larry Benchimol and Magali Romero Sá**

This paper is part of a research project, Adolpho Lutz and the history of tropical medicine in Brazil, aimed at producing a critical and annotated edition of the scientific work and unpublished correspondence of the Brazilian scientist. It also intends to produce a biographic essay and a review of the history of tropical medicine during Lutz’s professional years.

A world pioneer in the scientific study of the microbial agent of leprosy and of its clinical and epidemiological aspects, Lutz emphatically defended the theory that the disease was transmitted by mosquitoes.

During his early study years in Switzerland and Germany, Adolfo Lutz (1855 to 1940) had already published papers in zoology and clinical and therapeutic practices. The fundamental study he later developed on Ancylostoma duodenale, published in Leipzig in 1885, contributed to the Brazilian helminthological research agenda in
syntony with the theoretical and methodological practices of German, British and French microbiologists and parasitologists of the time.

In 1885 to 1886, Lutz travelled to Hamburg to study with Paul Gerson Unna, one of the foremost German dermatologists. During that time, he investigated the morphology of germs related to several skin diseases, having even proposed a new classification for the agent of leprosy, removing it from the genus *Bacillus* and re-classifying it as *Coccothrix leprae*. Lutz’s study was facilitated by a new staining method, developed by him and improved by Unna. In 1889, the German dermatologist recommended his most prominent student as physician-in-chief of the Leprosarium at Molokai Island, Hawaii. Lutz worked there for nine months, after which he continued his research on leprosy in his own private clinic for more than a year. From Hawaii, he moved to California in 1892, spending some months there, before his return to Brazil.

In 1893, Lutz assumed the direction of the Bacteriological Institute in São Paulo. By that time, medical entomology—especially the study of mosquitoes—became one of his main interests. He developed research in both urban and forest yellow fever (which he foresaw), as well as in forest malaria and the transmission of leprosy by mosquitoes. Since his time in Hawaii, Lutz had been fostering the notion that leprosy was transmitted by mosquitoes. He continued research on the subject when he moved to Instituto Oswaldo Cruz in 1908, having passionately defended this idea in scientific meetings and medical commissions until his death in 1940.

As the world witnesses ever-increasing rates of tuberculosis, particularly of drug-resistant strains affecting some of society’s most marginalized individuals, policy makers and legislators may again visit the statute books in order to strengthen their armamentarium of tools to protect public health. This paper assesses the evidence in support of the sanction to detain those with tuberculosis who are perceived as posing a public health threat, and shows that little research has been conducted to inform policy, probably because traditional epidemiological methods used to assess the impact of interventions are not feasible.—Author’s Abstract


In order to evaluate the measures taken against Hansen’s diseases during the colonial era in Korea, from 1910–1945, I analyzed both Korean and Japanese materials and carried out field research. The Korean government-general established a hospital in 1916 and executed measures against Hansen’s disease. These efforts can be divided into three periods. At first they started as a part of colonial policy. Then, in the middle period, with the change of Japanese policy on Hansen’s disease, a Korean association was established and the Hansen’s Disease Prevention Act was issued in Korea, aiming at the compulsory isolation of lepers. In the later period, during the war, the inmates were forced into an extremely severe environment and deprived of their human rights. My study shows that their policies changed greatly with the passage of time. Though they started them to relieve the suffering of the lepers in the beginning, they turned to be compulsory isolation of the patients in the later period and to the violation of their human rights.—Author’s Abstract

Muller, P., Frederic, M., Salzer, B., and Strobel, M. [Leprosy in Guadeloupe (French West Indies): declining disease,

INTRODUCTION: Endemic for nearly three centuries, leprosy is declining in Guadeloupe: its prevalence has decreased by 75 p. 100 over the last decade. Because it has become rare, it may well be overlooked. PATIENTS AND METHODS: Retrospective study of all the new cases of leprosy diagnosed in Guadeloupe from May 1996 to May 2001. RESULTS: In 10 cases of the 41 reported in this study, diagnosis had been delayed by more than 6 months. Nine of these 10 cases presented with classical clinical signs. The mean delay before diagnosis in these 10 cases was of 22 months (range: 7 to 36 months); the mean number of consultations with a physician before the final diagnosis was of 3.2 (range: 2–8). The mean age at the time of diagnosis in patients in whom diagnosis was delayed was significantly greater than those in whom diagnosis was confirmed rapidly (55 vs 37 years). DISCUSSION: In Guadeloupe, one patient out of 4 presenting with leprosy is diagnosed with a delay of more than 6 months, despite a classical clinical presentation. This is deleterious to the patients and health economics. The patients in whom diagnosis was delayed were older. This epidemiological tendency appears inherent to this form of “residual leprosy”. The present rareness of the disease is responsible for a lack of knowledge of the disease by the physicians through lack of experience. This phenomenon is also observed for syphilis and measles. There is a real risk of underestimation or erroneous diagnosis.—Authors’ Abstract

Chemotherapy


INTRODUCTION: The side effects of rifampicin due to an immunological mechanism are rare and usually observed during discontinued treatment or administration of high doses. OBSERVATIONS: An immediate hypersensitivity reaction with anaphylactic manifestations and increase in IgE occurred in a 39-year-old man suffering from resistant tuberculosis. The reaction occurred within the first hour following a low dose of rifampicin administered in a desensitization attempt, the outcome of which was favorable after administration of corticosteroids and antihistamines. A type II hypersensitivity reaction occurred in a 76-year-old male patient in the form of thrombopenia on D76 of a twice weekly treatment, diagnosed because of hemoptysis with normalization of platelet level on withdrawal of rifampicin. An immune complex hypersensitivity reaction was responsible for hepato-renal failure on D68 of twice weekly treatment and required permanent withdrawal of rifampicin and dialysis, which led to subsequent improvement. COMMENTS: These clinical cases illustrate the variability of the hypersensitivity mechanisms observed with rifampicin, the difficulty in imputability tests and methods for immunological confirmation, the interest of continuous treatment which avoids a certain number of these accidents, and that of desensitization during immediate hypersensitive reactions which permits the continuation this major anti-tuberculosis drug.—Authors’ Abstract


BACKGROUND: Mycobacterium avium-intracellulare (MAC) causes progressive lung disease. Recommended treatment regimens include a macrolide and a rifamycin, but drug intolerance and relapse
after treatment is completed often limit successful therapy. METHODS: Consecutive individuals referred for treatment of MAC lung disease were treated with a regimen that included either clarithromycin, 500 mg bid, or azithromycin, 250 mg/d, on weekdays; ethambutol, 15 mg/kg/d; and clofazimine, 100 mg/d. The intention was to treat patients for a minimum of 12 months. The diagnosis of MAC lung disease was confirmed by multiple positive sputum culture findings in patients with typical symptoms and radiologic findings. RESULTS: Thirty patients (27 women and 3 men; mean age, 70 ± 9.4 years [S.D.]) were treated. A total of 22 of the patients reported adverse effects from clarithromycin or azithromycin. Intolerance of clarithromycin resulted in the withdrawal of four patients before sputum conversion. The remaining patients continued treatment for an average of 10 months, and sputum findings converted to negative in all 26 patients (87%). One patient died of unrelated causes while still receiving therapy, and five patients (19%) relapsed an average of 17 months after treatment was completed. CONCLUSIONS: Treatment with a macrolide, ethambutol, and clofazimine was successful in 20 of 30 patients (67%) with MAC lung disease and is a reasonable alternative to rifamycin-containing regimens.—Authors’ Abstract


A series of 2-acetyl and 2-benzoyl-6(7)-substituted quinoxaline 1,4-di-N-oxide derivatives were synthesized and evaluated for in vitro antituberculosis activity. The results show that 2-acetyl-3-methylquinocxaline 1,4-di-N-oxide derivatives with chlorine, methyl or methoxy group in position 7 of the benzene moiety (compounds 2, 4 and 6, respectively) and unsubstituted (3) have good antitubercular activity, exhibiting EC(90)/MIC values between 0.80 and 4.29. In conclusion, the potency, selectivity and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity.—Authors’ Abstract


 OBJECTIVE: To report a case of acute pancreatitis associated with dapsone use. CASE SUMMARY: An 87-year-old white man was prescribed dapsone for dermatitis herpetiformis. Four weeks later, he developed acute abdominal pain requiring hospitalization. The patient had elevated serum amylase and lipase levels. Laboratory test results for other possible etiologies were negative. His symptoms resolved when dapsone was discontinued. Dapsone was reintroduced for exacerbation of dermatitis herpetiformis 4 months later. The patient again had severe abdominal pain with high amylase and lipase levels. Again, symptoms resolved following dapsone discontinuation. DISCUSSION: Only 1 other case of pancreatitis associated with dapsone was found in a MEDLINE search of the literature (1966–June 2003) using the key terms dapsone and pancreatitis. An objective causality assessment revealed dapsone to be a probable cause of acute pancreatitis, based on the Naranjo probability scale. Drugs should always be considered as causative factors for pancreatitis in patients without known risk factors. Dapsone is increasingly used as a second line of treatment of Pneumocystis carinii pneumonia (PCP). The recognition of dapsone-induced pancreatitis is of particular importance in these patients. CONCLUSIONS: While dapsone is traditionally used for the treatment of leprosy and dermatitis herpetiformis, its use for PCP prophylaxis, malaria, brown recluse spider bites, and acne is not uncommon. Pancreatitis is an uncommon adverse effect of dapsone, and greater awareness of this association will prompt a high index of suspicion in an appropriate clinical setting. Further reporting of cases and clinical research of drug-induced pancreatitis is indicated.—Authors’ Abstract

Mycobacterial infections have recently attracted significant attention from international health agencies due to the resurgence of these diseases worldwide. This review summarizes the recent work directed towards the identification of new anti-tuberculosis agents that act by inhibiting mycobacterial cell wall polysaccharide biosynthesis.—Author’s Abstract


Thalidomide, (1), has made a remarkable comeback from its days of a sedative with teratogenic properties due to its ability to selectively inhibit TNF-alpha, a key pro-inflammatory cytokine and its clinical benefit in the treatment of cancer. Thalidomide contains one chiral center and is known to be chirally unstable under in vitro and in vivo conditions. It has been hypothesized that different biological properties are associated with each isomer. Thus, chirally stable analogues of thalidomide, alpha-fluorothalidomide, (3) and alpha-fluoro-4-aminothalidomide (4) were prepared by electrophilic fluorination. analogue 3 was found to be cytotoxic and did not inhibit TNF-alpha production in LPS stimulated hPBMC below toxic concentrations. On the other hand, 4 was non-cytotoxic at the tested concentrations and was found to be 830-fold more potent than thalidomide as TNF-alpha inhibitor.—Authors’ Abstract


Aims: Our aim was to investigate the effects of rifampicin on the pharmacokinetics and pharmacodynamics of nateglinide, a novel short-acting antidiabetic drug. METHODS: In a randomized crossover study with two phases, 10 healthy volunteers took 600 mg rifampicin or placebo orally once daily for 5 days. On day 6 of both phases, they ingested a single 60 mg dose of nateglinide. Plasma nateglinide and blood glucose concentrations were measured for up to 7 h postdose. RESULTS: Rifampicin decreased the mean AUC(0,7 h) of nateglinide by 24% (range 5–53%; p = 0.0009) and shortened its half-life (t(1/2)) from 1.6 to 1.3 h (p = 0.001). However, the peak plasma nateglinide concentration (Cmax) remained unchanged. The AUC(0,7 h) of the M7 metabolite of nateglinide was decreased by 24% (range 5–53%; p = 0.0009) and its t(1/2) was shortened from 2.1 to 1.6 h by rifampicin (p = 0.008). Rifampicin had no significant effect on the blood glucose-lowering effect of nateglinide. CONCLUSIONS: Rifampicin modestly decreased the plasma concentrations of nateglinide proba-
bly by inducing its oxidative biotransformation. In some patients, rifampicin may reduce the blood glucose-lowering effect of nateglinide.—Authors’ Abstract


OBJECTIVE: Our objective was to investigate the effect of rifampin (INN, rifampicin) on the pharmacokinetics and pharmacodynamics of gliclazide, a sulfonylurea antidiabetic drug. METHOD: In a randomized 2-way crossover study with a 4-week washout period, 9 healthy Korean subjects were treated once daily for 6 days with 600 mg rifampin or with placebo. On day 7, a single dose of 80 mg gliclazide was administered orally. Plasma gliclazide, blood glucose, and insulin concentrations were measured. RESULTS: Rifampin decreased the mean area under the plasma concentration-time curve for gliclazide by 70% (p <0.001) and the mean elimination half-life from 9.5 to 3.3 hours (p <0.05). The apparent oral clearance of gliclazide increased about 4-fold after rifampin treatment (p <0.001). A significant difference in the blood glucose response to gliclazide was observed between the placebo and rifampin phases. CONCLUSION: The effect of rifampin on the pharmacokinetics and pharmacodynamics of gliclazide suggests that rifampin affects the disposition of gliclazide in humans, possibly by the induction of cytochrome P450 2C9. Concomitant use of rifampin with gliclazide can considerably reduce the glucose-lowering effects of gliclazide.—Authors’ Abstract


Two novel alkaloids, named manadomanzamines A (1) and B (2), were isolated from an Indonesian sponge Acanthostylophora sp. (Haplosclerida: Petrosiidae). Their structures were elucidated and shown to be a novel organic skeleton related to the manzamine type alkaloids. Their absolute configuration and conformation were determined by CD, NOESY, and molecular modeling analysis. The microbial community analysis for the sponge that produces these unprecedented alkaloids has also been completed. Manadomanzamines A (1) and B (2) exhibited strong activity against Mycobacterium tuberculosis (Mt) with MIC values of 1.9 and 1.5 μg/mL, respectively. Manadomanzamines A and B also exhibit activities against human immunodeficiency virus (HIV-1) and AIDS opportunistic fungal infections.—Authors’ Abstract


The thiourea isoxyl (thiocarlide; 4,4′-diisooamyloxydiphenylthioure) is known to be an effective anti-tuberculosis drug, active against a range of multidrug-resistant strains of Mycobacterium tuberculosis and has been used clinically. Little was known of its mode of action. We now demonstrate that isoxyl results in a dose-dependent decrease in the synthesis of oleic and, consequently, tuberculostearic acid in M. tuberculosis with complete inhibition at 3 microl/mL. Synthesis of mycolic acid was also affected. The anti-bacterial effect of isoxyl was partially reversed by supplementing growth medium with oleic acid. The specificity of this inhibition pointed to a Delta9-stearoyl desaturase as the drug target. Development of a cell-free assay for Delta9-desaturase activity allowed direct demonstration of the inhibition of oleic acid synthesis by isoxyl. Interestingly, sterculic acid, a known inhibitor of Delta9-desaturases, emulated the effect of isoxyl
on oleic acid synthesis but did not affect mycolic acid synthesis, demonstrating the lack of a relationship between the two effects of the drug. The three putative fatty acid desaturases in the *M. tuberculosis* genome, desA1, desA2, and desA3, were cloned and expressed in *Mycobacterium bovis* BCG. Cell-free assays and whole cell labeling demonstrated increased Delta9-desaturase activity and oleic acid synthesis only in the desA3-overexpressing strain and an increase in the minimal inhibitory concentration for isoxyl, indicating that DesA3 is the target of the drug. These results validate membrane-bound Delta9-desaturase, DesA3, as a new therapeutic target, and the thioureas as anti-tuberculosis drugs worthy of further development.—Authors’ Abstract


An original, simple, specific, and rapid high-performance liquid chromatography assay for the determination of clofazimine in human plasma is presented. The procedure consists of extracting the drug and the internal standard (medazepam) from 0.5 mL plasma with dichloromethane/diisopropyl ether (1:1, v/v) at pH 3.0, after precipitating the proteins with methanol. The drugs were then quantitated on a reversed-phase C8 using a mobile phase consisting of a mixture of methanol/0.25 N sodium acetate buffer at pH 3.0 (74:26, v/v). The flow-rate and wavelength were set at 1 mL/min and 286 nm, respectively. The precision, linearity, and limit of quantitation of the method were within acceptable limits. The method was considered adequate and could be applied in studies involving blood level monitoring and pharmacokinetics in leprosy patients.—Authors’ Abstract


Anemia may result in tissue hypoxia which may induce or exacerbate symptoms of ischemia. Tissue hypoxia may however also result from the presence of hemoglobin with altered oxygen-binding characteristics. Drug-induced methemoglobinaemia in which oxygen is irreversibly bound to hemoglobin may complicate the use of some common drugs. This condition may result in severe tissue hypoxia, which is rapidly and cheaply reversed by methylene blue.—Authors’ Abstract


A 36-year-old male patient suffered from therapy resistant sarcoidosis with longstanding contractures, myopathy, skin lesions and pulmonary changes. Low-dose therapy with thalidomide (50 mg/day) was well tolerated, and the patient rapidly improved. Thalidomide was effective for muscular, cutaneous, and pulmonary involvement in our patient. This is the first report on the efficacy of thalidomide in muscle sarcoidosis. Therefore, thalidomide may become a second-line agent in patients with severe muscle and skin involvement, but further studies are warranted.—Authors’ Abstract


Pyrazinamide is an important sterilizing drug that shortens tuberculosis (TB) therapy. However, the mechanism of action of pyrazinamide is poorly understood because of its unusual properties. Here we show that pyrazinoic acid, the active moiety of pyrazinamide, disrupted membrane energetics and inhibited membrane transport function in *Mycobacterium tuberculosis*. The preferential activity of pyrazinamide
against old non-replicating bacilli correlated with their low membrane potential and the disruption of membrane potential by pyrazinoic acid and acid pH. Inhibitors of membrane energetics increased the antituberculous activity of pyrazinamide. These findings shed new light on the mode of action of pyrazinamide and may help in the design of new drugs that shorten therapy.—Authors’ Abstract

Clinical Sciences


OBJECTIVE: To study and evaluate the efficacy and safety of recombinant human interleukin-2 (IL-2) in the treatment of pulmonary tuberculosis. METHODS: Two hundred and nine cases with re-treated Mycobacterium tuberculosis-positive pulmonary tuberculosis were randomly divided into a trial group (106 cases, treated with 3PaZ (TH)L(2)VE(AK) + IL-2/4PaL(2)V) and a control group (103 cases, treated with 3PaZ(TH)L(2)VE(AK)/4PaL(2)V). The efficacy of 203 cases was available for evaluation when the course was completed (trial group 103 cases, control group 100 cases). RESULTS: The sputum smear-negative conversion rates at the 1st and the 2nd month of therapy were 33.3% and 69.4% in the trial group, and 7.2% and 44.9% in the control group (p <0.01). At the completion of the therapy, the X-ray resolution rates were 64.1% and 36.0% respectively for the trial and the control groups, the difference being significant (p <0.001). There were significant differences in CD(4) T cells, the ratio of CD(4)/CD(8) and NK cells between the two groups (p <0.01). The level of soluble interleukin-2 receptor (sIL-2R) was significantly different between the two groups after treatment for 3 months (p <0.05). IL-2 associated side effects were rare and mild. CONCLUSION: As an effective and relatively safe biological agent, IL-2 can be added to the standard chemotherapy for pulmonary tuberculosis.—Authors’ Abstract


During the last decade, annual tuberculosis (TB) case-notification rates increased 4-fold, to >4000 cases/100,000 person-years, in the study workforce, among whom prevalence of human immunodeficiency virus (HIV) was 30% in 2000. Three separate cohort studies, totalling 6454 HIV-negative participants, were combined and analyzed for time trends. Observed incidence of TB varied between 962 (1991–1994) and 1589 (1999–2000) cases/100,000 person-years (p = 0.17, test for trend). There was, however, a progressive increase in age, and, for each period, older age was associated with increased incidence rates of TB (p <0.001). Having adjusted for age differences, there was no significant association between incidence of TB and calendar period (p = 0.81, test for trend). Relative to 1991–1994, multivariate-adjusted incidence-rate ratios were 0.94, for 1995–1997, 0.96, for 1998–1999, and 1.05, for 1999–2000. Preventing a secondary epidemic of TB among HIV-negative individuals may be achievable with conventional means, even in settings with a high burden of HIV-associated TB.v.—Authors’ Abstract

This study identifies possible obstacles to the early diagnosis of leprosy. Semi-structured interviews were held with 40 patients at a secondary health service in upstate Sao Paulo, Brazil. The data concerning the sample were: 75% males, age range 13 to 76 years, 85% with elementary school education, 85% multibacillary. Skin lesions associated with sensory alterations had been noticed by 55% of the patients; 32.5% of the patients had been misdiagnosed as having conditions other than leprosy. The diagnosis was made 1 year after the awareness of signs/symptoms in 55% of the patients. In this group, 54% had impairment grade 1, while 23% had no disabilities. Forty-five percent of all patients interviewed had some information about the disease prior to diagnosis. Eleven patients (27.5%) had previous contact with leprosy patients, but this did not prevent late diagnosis in 64%. After the disease was confirmed, about half of the interviewed patients (47.5%) showed mainly positive feelings due to the prospect of treatment and cure. Our results suggest that misdiagnoses and unawareness of the disease were the main factors that influenced the delayed diagnosis. We consider the effective involvement of various segments of society, particularly the integration and partnership of the public health services and health education centres to be valuable tools for the planning and execution of educational activities directed at risk groups and the community.—Authors’ Abstract


Leprosy is one of the most common diseases of the peripheral nerves. In some cases there is only neural involvement without skin changes (neuritic form). The neuropathy has often a distal stocking and glove distribution with thermal and pinprick anesthesia and preservation of proprioception. There is no weakness, the tendon reflexes may be preserved and sometimes the nerves are thickened. We reported 17 patients with a predominantly small-fiber polyneuropathy due to leprosy. All patients had distal temperature and pain anesthesia with different individual variations. The tendon reflexes were normal in seven patients and in eight there was thickening of the nerves. The nerve conduction was normal in three patients. Sural nerve biopsy consisted of: 1) inflammatory infiltrates, 2) vacuolated “foamy” cells, 3) fibrosis of endoneurium, perineurium, and epineurium, 4) partial or total loss of nerve fibers, 5) large number of bacilli. We concluded that in countries where leprosy is frequent, nerve biopsy is an obligatory procedure in patients with predominantly small-fiber polyneuropathy.—Authors’ Abstract


A middle-aged HIV infected man receiving treatment for pulmonary tuberculosis, presented with a febrile illness along with evanescent, erythematous nodular lesions all over the body. On examination, he had features suggestive of lepromatous leprosy with lesions of erythema nodosum leprosum. In addition, there were multiple small, circumscribed areas of slack skin, clinically and histopathologically suggestive of anetoderma. Both leprosy and HIV infection are known to give rise to lesions of anetoderma. Pathogenesis of anetoderma in these infectious conditions is discussed.—Authors’ Abstract


Concomitant tuberculosis and leprosy is uncommon, even in endemic countries. We report a patient with borderline lepromatous leprosy and type 1 reversal reaction initially diagnosed while the patient was undergoing treatment for pulmonary tuberculosis. The diagnosis was on the basis of characteristic histopathology and Fite-Farraco stain.—Authors’ Abstract

In leprosy, sensory action potentials (SAPs) may be normal in spite of clinical sensory loss. This may result from the early involvement of small nerve fibers, which have potentials that are not detected in routine studies. To evaluate this possibility, we used a near-nerve recording technique that records potentials from nerve fibers as small as 4 to 6 microm in diameter. We hypothesized that this technique might increase the sensitivity of nerve conduction studies in detecting leprosy neuropathy. We found the technique to be useful for recording conduction abnormalities in recently diagnosed patients, including those with preserved sensation, suggesting that axonal loss may be the underlying mechanism. Contrary to our hypothesis, however, recording the late SAP components did not improve the sensitivity of nerve conduction studies. We suggest that the late components having normal conduction velocities may be generated by either regenerating or remyelinating abnormal fibers, which have an electrophysiological behavior similar to that of normal 4 to 6-microm-diameter fibers.—Authors’ Abstract


Mycobacterial infections are grouped into infections caused by *M. tuberculosis* and those caused by the atypical mycobacterial organisms. Tuberculosis is a systemic disease, with cervical lymphadenitis of the head and neck being the most common extrapulmonary manifestation of the disease. It is important to use imaging, histopathologic examination, and culture to differentiate tuberculosis from atypical mycobacterial infections, because treatments differ. Tuberculosis is best treated as a systemic disease, with anti-tuberculosis medication. The atypical infections can be addressed as local infections and are amenable to surgical therapy.—Authors’ Abstract


BACKGROUND: Disabilities constitute the main problem of leprosy. It is important to identify risk factors involved, so it can be possible the prone patients be followed-up more carefully. OBJECTIVES: To determine if the presence of thick and/or painful peripheral nerves at diagnosis correlates with disabilities already present at the initial examination, as well as with subsequent development of neuritis, during and after multidrug therapy. METHODS: One hundred and three patients with multibacillary forms of leprosy were studied, and we noted the presence of compromised peripheral nerves at diagnosis, the disability grade before treatment (DGBT), and the occurrence of neuritis episodes during and after multibacillary multidrug therapy. RESULTS: The detection of affected peripheral nerves at diagnosis correlated statistically (p <0.005) with the occurrence of disabilities (DGBT >0). It also correlated significantly with the development of neuritis in the follow-up (average of 64.6 months from diagnosis, during and after multidrug therapy). CONCLUSIONS: We emphasize the need of a good examination of peripheral nerve trunks in multibacillary patients at the diagnosis, in order to improve the detection of disabilities already present, and specially to prevent further disabilities. Healthy professionals who deal with leprosy patients must be aware to the initial neurological impairments because those patients are more susceptible to the occurrence of neuritis and neurological sequelae. —Anais Brasileiros de Dermatologia


La tuberculosis es una enfermedad infec- tocontagiosa crónica provocada por el *Mycobac-
cobacterium tuberculosis, el Mycobacterium bovis y, en ciertas condiciones, por el bacilo de Calmette-Guérín (BCG), cepa atenuada de Mycobacterium bovis.

A partir de la década del 80, hubo un incremento a nivel mundial de los pacientes afectados de tuberculosis pulmonar y extrapulmonar; este aumento en los índices de incidencia se vio asociado a la pandemia provocada por el virus HIV.

La tuberculosis cutánea corresponde al 1% de los casos de tuberculosis extrapulmonar. Esta forma de tuberculosis es de distribución mundial, pero predomina en regiones de clima húmedo y frío. Afecta predominantemente a personas de nivel socioeconómico bajo.

A continuación presentamos un caso de tuberculosis verrucosa cutánea dada su infrecuencia y hacemos una revisión bibliográfica sobre esta patología. —Revista Argentina de Dermatología

Ustianowski, A. P., and Lockwood, D. N.

PURPOSE OF REVIEW: Leprosy remains an important problem globally and leprosy patients may present to physicians outside leprosy endemic areas. We review the recent biological and clinical advances in leprosy.

RECENT FINDINGS: Sequencing the genome has been a major biological advance and will open up new possibilities for research. The three cardinal criteria (anaesthetic skin patches, thickened nerves and acid-fast bacilli in skin smears) have not yet been bettered. Multidrug therapy for leprosy is highly effective with low relapse rates though the optimal duration of therapy for multibacillary patients is unclear. Nerve damage remains a significant problem (in some series only 50% responding to steroid therapy). New treatments for leprosy reactions are needed. Stigma remains a problem but is being combated by patient groups.

SUMMARY: Far from being eliminated as a public health problem, leprosy still causes a considerable long-term morbidity in both the developing and developed world. New treatments for leprosy reactions are needed and the optimal length of multidrug therapy required further research.—Authors’ Abstract

Immunopathology

Feng, C. G., Scanga, C. A., Collazo-Custodio, C. M., Cheever, A. W., Hiény, S., Caspar, P., and Sher, A.

To assess the role of Toll-like receptor (TLR) signaling in host resistance to Mycobacterium avium infection, mice deficient in the TLR adaptor molecule myeloid differentiation factor 88 (MyD88), as well as TLR2(−/−) and TLR4(−/−) animals, were infected with a virulent strain of M. avium, and bacterial burdens and immune responses were compared with those in wild-type (WT) animals. MyD88(−/−) mice failed to control acute and chronic M. avium growth and succumbed 9–14 wk postinfection. Infected TLR2(−/−) mice also showed increased susceptibility, but displayed longer survival and lower bacterial burdens than MyD88(−/−) animals, while TLR4(−/−) mice were indistinguishable from their WT counterparts. Histopathological examination of MyD88(−/−) mice revealed massive destruction of lung tissue not present in WT, TLR2(−/−), or TLR4(−/−) mice. In addition, MyD88(−/−) and TLR2(−/−) mice, but not TLR4(−/−), mice displayed marked reductions in hepatic neutrophil infiltration during the first 2 h of infection. Although both MyD88(−/−) and TLR2(−/−) macrophages showed profound defects in IL-6, TNF, and
IL-12p40 responses to *M. avium* stimulation *in vitro*, *in vivo* TNF and IL-12p40 mRNA induction was impaired only in infected MyD88(−/−) mice. Similarly, MyD88(−/−) mice displayed a profound defect in IFN-gamma response that was not evident in TLR2(−/−) or TLR4(−/−) mice or in animals deficient in IL-18. These findings indicate that resistance to mycobacterial infection is regulated by multiple MyD88-dependent signals in addition to those previously attributed to TLR2 or TLR4, and that these undefined elements play a major role in determining bacterial induced proinflammatory as well as IFN-gamma responses.—Authors’ Abstract


Live mycobacteria have been reported to signal through several pattern recognition receptors (PRR), among them toll-like receptor 4 (TLR4) and TLR2 *in vitro*. Here, we investigated the role of TLR4 in host resistance to *Mycobacterium bovis* (BCG) infection *in vivo*. *In vitro*, macrophages of TLR4 mutant C3H/HeJ mice infected with BCG expressed lower levels of TNF than controls, and TNF release was further decreased, although not completely absent, in the absence of TLR2. *In vivo*, TLR4 mutant C3H/HeJ and control C3H/HeOuJ mice were infected with BCG (2 × 10(6) CFU i.v.). Both TLR4 mutant and wild-type mice were able to control the infection and survived 8 months post-BCG infection. Macrophage activation with abundant acid-fast bacilli and expression of inducible nitric oxide synthase (iNOS) and MHC class II antigens was seen in both groups of mice. However, TLR4 mutant mice experienced an arrest of body weight gain and showed signs of increased inflammation, with persistent splenomegaly, increase in granuloma number and augmented neutrophil infiltration. Infection of TLR4-deficient mice with higher doses of BCG (1 and 3 × 10(7) CFU, i.v.) increased the inflammation in spleen and liver, associated with a transient, higher bacterial load in the liver. In summary, TLR4 mutant mice show normal macrophage recruitment and activation, granuloma formation and control of the BCG infection, but this is associated with persistent inflammation. Therefore, TLR4 signaling is not essential for early control of BCG infection, but it may have a critical function in fine tuning of inflammation during chronic mycobacterial infection.—Authors’ Abstract


SETTING: The success of *Mycobacterium tuberculosis* as a human pathogen depends on its ability to tolerate and perhaps manipulate host defense mechanisms. OBJECTIVE: To determine the induction of tumour necrosis factor-alpha (TNF alpha), a central mediator of immunity, by human monocytes infected with virulent *M. tuberculosis*, *M. leprae* and attenuated *M. bovis* BCG. DESIGN: Mycobacteria-induced cellular activation pathways of TNF alpha production was investigated using an inhibitor of protein tyrosine kinase (PTKs) and an inhibitor of mitogen-activated protein (MAP) kinases. RESULTS: TNF alpha production was significantly lower during infection with virulent *M. tuberculosis* than with BCG and this differential response was independent of mycobacterial viability. TNF alpha production involved the PTK and MAP kinase pathways. Reduced TNF alpha induction by *M. tuberculosis* was associated with a reduction in the extent and duration of phosphorylation of extracellular-signal-regulated kinases (ERK 1/2). Infection with *M. leprae* triggered low and transient ERK 1/2 activation as well as reduced phosphorylation of extracellular-signal regulated kinases (ERK 1/2). Infection with *M. leprae* triggered low and transient ERK 1/2 activation as well as low TNF alpha production. CONCLUSION: Maintenance of the differential response in both live and heat-killed preparations suggests that the reduced TNF alpha response associated with virulent mycobacteria is due to differences in the presence of components capable of triggering host pattern recognition re-
current Literature, Immunopathology


An array of mammalian phospho-specific antibodies was used to screen for a host response upon mycobacterial infection, reflected as changes in host protein phosphorylation. Changes in the phosphorylation state of 31 known signaling molecules were tracked after infection with live or heat killed Mycobacterium bovis BCG or after incubation with the mycobacterial cell wall component lipoarabinomannan (LAM). Mycobacterial infection triggers a signaling cascade leading to activation of stress-activated protein kinase and its subsequent downstream target, c-Jun. Mycobacteria were also shown to inhibit the activation of protein kinase C varepsilon and to induce phosphorylation of proteins not yet known to be involved in mycobacterial infection, such as the cytoskeletal protein alpha-adducin, glycogen synthase kinase 3beta, and a receptor subunit involved in regulation of intracellular Ca(2+) levels. The mycobacterial cell wall component LAM has been identified as a trigger for some of these modulation events.—Authors’ Abstract


This study explores the potential of the amplified ribosomal DNA restriction analysis (ARDRA) for intra- and interspecies identification of the genus Mycobacteria. A set of primers was used to amplify part of the 16S and 23S rDNA as well as the 16S–23S rDNA spacer from 121 isolates belonging to 13 different mycobacterial species. Restriction analysis was carried out with five different restriction enzymes, namely CfoI, HaeIII, Rsal, MspI and TaqI. Restriction digestion of the PCR product using CfoI enabled differentiation between 9 of the 13 mycobacterial species, whereas the remaining four enzymes differentiated between 7 of these 13 species. None of the five enzymes distinguished between different isolates of Mycobacterium tuberculosis or between species within the M. tuberculosis complex i.e., M. tuberculosis, M. bovis, M. bovis BCG and M. africanum. Although ARDRA analysis of the 16S–23S rDNA does not seem to have a potential for intraspecies differentiation, it has proven to be a rapid and technically relatively simple method to recognize strains belonging to the M. tuberculosis complex as well as to identify mycobacterial species outside this complex.—Authors’ Abstract


PURPOSE OF REVIEW: Exposure to certain environmental microorganisms can promote the induction of T regulatory cells via the innate immune system. This review explores the possibility that reduced exposure to such organisms is leading to increased immunoregulatory disorders in a subset of individuals in whom this regulatory T-cell-inducing pathway is less efficient. We concentrate on mycobacteria and on asthma, because these are well documented. RECENT FINDINGS: The blood cells of the children of farmers, who are partly protected from allergies, express increased levels of messenger RNA encoding CD14 and TLR2, and polymorphisms of CD14 are linked to allergic manifestations in some studies. Polymorphisms of TLR2 (which recognizes mycobacterial components in concert with CD14) are involved in the pattern of response to mycobacteria, and in the type of leprosy that develops. Similarly, polymorphisms of Nramp1, which affect the response to mycobacteria, are linked with the diseases of immunodysregulation that are increasing in parallel with allergic disorders. Moreover, congenic
mice bearing different variants of Nramp1 differ in their allergic responses. These parallels are suggestive, in view of the observation that a saprophytic environmental mycobacterium is a potent inducer of regulatory T cells, and has shown significant effects in several phase I/II studies in man.

SUMMARY: The components of the innate immune system that are involved in responses to mycobacteria overlap with those implicated in allergic disorders. Polymorphisms might define the subset of individuals who develop immunoregulatory disorders. Understanding the role of the innate immune system will facilitate the design of clinical trials using microbial products.—Authors’ Abstract


Interferon γ is believed to be crucial for host defense against many infections. To test the hypothesis that a polymorphism in the gene for interferon γ (IFNG) is associated with susceptibility to tuberculosis, we did two independent investigations. In a case-control study of 313 tuberculosis cases, we noted a significant association between a polymorphism (+874A→T) in IFNG and tuberculosis in a South African population (p = 0.0055). This finding was replicated in a family-based study, in which the transmission disequilibrium test was used in 131 families (p = 0.005). The transcription factor NFκB binds preferentially to the +874T allele, which is over-represented in controls. This preferential binding suggests that genetically determined variability in interferon γ and expression might be important for the development of tuberculosis. —Tropical Diseases Bulletin


Phagocytosis by macrophages represents the early step of the mycobacterial infection. It is governed both by the nature of the host receptors used and the ligands exposed on the bacteria. The outermost molecules of the nonpathogenic Mycobacterium smegmatis were extracted by a mechanical treatment and found to specifically and dose-dependently inhibit the phagocytosis of both M. smegmatis and the opportunistic pathogen M. kansasii by human macrophages derived from monocytes. The inhibitory activity was attributed to surface lipids because it is extracted by chloroform and reduced by alkaline hydrolysis but not by protease treatment. Fractionation of surface lipids by adsorption chromatography indicated that the major inhibitory compounds consisted of phospholipids and glycopeptidolipids (GPLs). Mass spectrometry and nuclear magnetic resonance spectroscopy analyses, combined with chemical degradation methods, demonstrated the existence of a novel family of GPLs that consists of a core composed of the long-chain tripeptidyl amino-alcohol with a di-O-acetyl-6-deoxytalosyl unit substituting the allo-threoninyl residue and a 2-succinyl-3,4-di-O-CH3-rhamnosyl unit linked to the alaninol end of the molecules. These compounds, as well as diglycosylated GPLs at the alaninol end and de-O-acylated GPLs, but not the non-serovar-specific di-O-acetylated GPLs, inhibited the phagocytosis of M. smegmatis and M. avium by human macrophages at a few nanomolar concentration without affecting the rate of zymosan internalization. At micromolar concentrations, the native GPLs also inhibit the uptake of both M. tuberculosis and M. kansasii. De-O-acylation experiments established the critical roles of both the succinyl and acetyl substituents. Collectively, these data provide evidence that surface-exposed mycobacterial glycoconjugates are efficient competitors of the interaction between macrophages and mycobacteria and, as such, could represent pharmacological tools for the control of mycobacterial infections.—Authors’ Abstract
Viveiros, M., Leandro, C., and Amaral, L.

The demonstration of the existence of active efflux pumps in mycobacteria raises the question of whether or not these can increase in number and activity rendering wild-type mycobacteria increasingly resistant to a given antibiotic. This could be a mechanism by which mutated resistant strains become better fit to the selective environment. Mycobacterium tuberculosis genome analysis reveals several genes encoding putative drug efflux pumps. During the course of tuberculosis chemotherapy many of these pumps might play a role in the survival of the mycobacterial populations. Compounds capable of inactivating these pumps could improve anti-tuberculous therapeutics.—Authors’ Abstract

Immunopathology (Leprosy)


See Current Literature, Molecular and Genetic Studies, p. 112.

Shankarkumar, U., Ghosh, K., Badakere, S., and Mohanty, D.

Convincing results on HLA Class II associations have been reported, however data on HLA class I association are limited and inconsistent from studies in Leprosy. We present here the HLA A, B, and C allele distribution by molecular high resolution PCR-SSOP technique in 32 leprosy patients compared with the 67 controls, from the same ethnic background. The significant results from the present study were a significant increase in frequency of HLA A*0206, A*1102, B*4016, B*5110, Cw*0407, and Cw*0703 was observed when compared to controls. A striking decrease in the frequency of HLA A*0101, Cw*04011, and Cw*0602 leprosy patients was observed when compared to the controls. Further haplotype A*1102-B*4006-Cw*1502 was significantly increased among the lepromatous leprosy patients when compared to the controls. It seems that HLA class I alleles play vital roles in disease association/pathogenesis with leprosy among Indians.—Authors’ Abstract

Sridevi, K., Khanna, N., Chattree, V., Pal, P. C., Haq, W., and Rao, D. N.

Mycobacterium leprae, the causative agent of leprosy resides and multiplies within the host monocytes and macrophages, thereby evading host immune system. Cell-mediated immune response (CMI) plays a vital role as evidenced from the high CMI in BT/TT (borderline and tuberculoid) patients and conversely low in BL/LL (borderline and lepromatous) patients. In the present study, an attempt was made to immunomodulate the anergized T cells of lepromatous leprosy patients by presenting the mycobacterial antigen in combination with T cell adjuvant, murabutide (active analog of muramyl‘ dipeptide, MDP-BE) and a Trat peptide (T cell epitope of Integral membrane protein (Trat) from Escherichia coli) in particulate form (liposomes) or soluble form (media). PBMNC
of normal, BT/TT and BL/LL were stimulated in vitro with five mycobacterial antigens (Ag) in the following formulations, Ag, Ag+murabutide, Ag+murabutide+Trat peptide either in liposomes or in medium. All the five antigen(s) when delivered in liposomes containing murabutide and Trat peptide showed a very high lymphoproliferative response (p <0.001) in all the three groups. IFN-gamma and IL-2 were significantly (p <0.001) high in these culture supernatants compared to IL-10 and IL-4 confirming a shift from CD4+Th2 to Th1 response in leprosy patients with particulate mode of antigen presentation. Interestingly, PBMNC derived from lepromatous patients also showed consistent T cell proliferation with all the formulations. Further, the mechanism of liposomal processing of antigens was studied using different inhibitors that interfere at different stages of antigen presentation. Results indicate that this study may pave way for an immunotherapeutic approach for reverting the anergic T cells of lepromatous patients to proliferating T cells with the release of Th1 cytokines thereby restoring the CMI response in these patients.—Authors’ Abstract


The immune response in reversal reaction, (RR) and in erythema nodosum leprosum (ENL) is characterized in vitro by an enhancement in lymphocyte blast transformation against M. leprae. As thalidomide is an effective treatment for ENL, this study assessed the effect of this drug on these phenomena. Mononuclear cells from patients attending the clinic at ALERT and from healthy staff were cultured for 5 days with integral M. leprae (IMl), or a modified Dharmendra antigen (Dhar), or PPD from M. tuberculosis. In one set of cultures, thalidomide was added once at the initiation of the culture; in the other set thalidomide was added a second time (2×), 18 h prior to harvesting the cells. The mononuclear cells, in the absence of thalidomide, from healthy staff, borderline tuberculoid patients (BT) and BT patients in RR (BT/RR) incorporated [3H]-thymidine best when cultured with PPD > Dhar > M. leprae. The cells from patients with ENL did not respond well to the M. leprae antigens. Thalidomide (2×) enhanced proliferation to Dhar in the BTRR group (Wilcoxon signed rank test, p <0.05). No significant changes occurred for the other groups. Comparing PPD-stimulated cells treated with thalidomide once to those treated with thalidomide twice, thalidomide (2×) suppressed incorporation of [H3]-thymidine by the PPD-stimulated (p <0.05) as well as IMl-stimulated (p <0.05) cells in the healthy


We explored the prognostic value of in situ cytokine patterns in 39 patients with single-skin-lesion paucibacillary leprosy before single-dose therapy, with 3 years of follow-up. Interferon (IFN)-gamma, interleukin (IL)-12, IL-10, IL-4, tumor necrosis factor (TNF)-alpha, and macrophage inflammatory protein (MIP)-1alpha mRNA was quantified in skin biopsy samples at diagnosis, and Mycobacterium leprae DNA was detected in 51.4% of cases. Type 1 immunity predominance with measurable IFN-gamma and undetectable IL-4, which is indicative of effective cell-mediated immunity, is compatible with both the reversal reactions (33.3%) and the resolution of lesions (64.1%) observed. A positive correlation between IL-12 and IFN-gamma indicated type 1 polarization via IL-12. The TNF-alpha/MIP-1alpha correlation implied the TNF-alpha induction of chemokines, which is important for granuloma formation. Positive correlations between key regulatory cytokines-IL-10 and IFN-gamma, IL-10 and IL-12, and IL-10 and TNF-alpha suggests that there may be some level of an intraleisional pro- or anti-inflammatory mechanism essential in avoiding immunopathology.—Authors’ Abstract
staff group. In the Dhar-stimulated cells from the healthy staff thalidomide significantly suppressed TNF-alpha (p <0.05). A mixed effect was seen within and between the other groups, but there was a trend for thalidomide to suppress TNF-alpha induced by the *M. leprae*, Dhar and PPD antigens.—Authors’ Abstract

**Immunopathology (Tuberculosis)**


Mitochondria are at the center of molecular events involved in energy production, cell survival and apoptosis. Mitochondrial membrane potential (Deltapsim) is maintained by cellular catabolic reactions and the electron transport chain of which cytochrome c is a constituent, whereas the proton leak pathway, ATP synthesis and turnover consume it. Mitochondrial alterations such as a drop in Deltapsim, swelling and cytochrome c release have been observed in apoptosis. However, there is a paucity of information concerning mitochondrial function in the course of intracellular infections, a process that must certainly induce stress on the host cell. This work analyses the effect that two strains of mycobacteria of opposing virulence have on the mitochondria of murine macrophages in the early stages of infection. It was found that infection of J774 cells with both *Mycobacterium tuberculosis* H37Ra and *M. tuberculosis* H37Rv readily induced changes in Deltapsim as well as in mitochondrial morphology at the ultrastructural level. In addition, an increase in cytosolic ATP was found at 24 hr post infection with both strains of *M. tuberculosis*. Interestingly, only *M. tuberculosis* H37Rv was able to induce cytochrome c release from mitochondria to the cytosol, thus suggesting the occurrence in *M. tuberculosis* H37Rv of a specific factor(s) capable of regulating cytochrome c translocation. The precise role of cytochrome c release in the context of a mycobacterial infection remains to be elucidated.—Authors’ Abstract


Peripheral blood mononuclear cells (PBMC) were obtained from culture-proven tuberculosis (TB) patients before and after 2 and 6 months of chemotherapy with a multi-drug regimen. PBMC were tested for cellular responses in antigen-induced proliferation and interferon-gamma (IFN-gamma) assays in response to complex mycobacterial antigens (whole cell *Mycobacterium bovis* BCG and *M. tuberculosis*, cell walls and short-term culture filtrate [ST-CF] of *M. tuberculosis*), fractionated ST-CF antigens (fractions F1–F10) and ESAT-6. The responses in TB patients before anti-TB treatment were low (median stimulation index (SI) = 1–7, median delta IFN-gamma = 0–12 U ml(–1), and percent responders = 13–67%) to all the antigenic preparations. Following the administration of anti-TB chemotherapy for 2 months, there were significant (p <0.05) improvements in the cellular responses (median SI = 9–76, median delta IFN-gamma = 3–70 U ml(–1), and percent responders = 33–100%) to most of the antigenic preparations. Following the administration of anti-TB chemotherapy for 2 months, there were significant (p <0.05) improvements in the cellular responses (median SI = 9–76, median delta IFN-gamma = 3–70 U ml(–1), and percent responders = 33–100%) to most of the antigenic preparations. However, concanavalin A-induced proliferation responses of PBMC from the same patients before and after 2 months of chemotherapy were high and comparable (median SI = 101 and 114, respectively, p >0.05, 100% responders). A further increase in IFN-gamma responses (median delta IFN-gamma = 14–250 U ml(–1) and percent responders = 43–100%) to mycobacterial antigens was observed in patients.
receiving chemotherapy for 6 months. Among the ST-CF fractions, F1 and F2 containing low molecular mass proteins resulted in the highest responses, whereas ESAT-6 showed responses comparable to these fractions only in a minority of the patients. HLA-DR typing of these patients showed heterogeneity in the expression of molecules encoded by HLA-DRB genes. These results show that effective chemotherapy restores cellular responses of TB patients to a large number of *M. tuberculosis* antigens, which could be useful in monitoring the efficacy of anti-TB treatment.—Authors’ Abstract


See Experimental Infections, p. 98


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The variation in sequence and length in the C-terminal region among members of the unique PE (Pro-Glu) and PPE (Pro-Pro-Glu) protein families of *Mycobacterium tuberculosis* is a likely source of antigenic variation, giving rise to the speculation that these protein families could be immunologically important. Based on in silico analysis, we selected a hypothetical open reading frame (ORF) encoding a protein belonging to the PPE family and having epitopes with predictably higher antigenic indexes. Reverse transcriptase PCR using total RNA extracted from *in vitro*-cultured *M. tuberculosis* H37Rv generated an mRNA product corresponding to this gene, indicating the expression of this ORF (Rv2430c) at the mRNA level. Recombinant protein expressed in *Escherichia coli* was used to screen the sera of *M. tuberculosis*-infected patients, as well as those of clinically healthy controls (*N* = 10), by enzyme-linked immunosorbent assay. The panel of patient sera comprised sera from fresh infection cases (category 1; *N* = 32), patients with relapsed tuberculosis (category 2; *N* = 30), and extrapulmonary cases (category 3; *N* = 30). Category 2 and 3 sera had strong antibody responses to the PPE antigen, equal to or higher than those to other well-known antigens, such as Hsp10 or purified protein derivative (PPD). However, a higher percentage of patients belonging to category 1, as opposed to clinically healthy controls, showed stronger antibody response against the PPE protein when probed with anti-immunoglobulin M (IgM) (71 vs 37.5%) or anti-IgG (62.5 versus 28.12%). Our results reveal that this PPE ORF induces a strong B-cell response compared to that generated by *M. tuberculosis* Hsp10 or PPD, pointing to the immunodominant nature of the protein.—Authors’ Abstract


Dihydrodipicolinate reductase (DHPR) catalyzes the reduced pyridine nucleotide-dependent reduction of the alpha,beta-unsaturated cyclic imine, dihydrodipicolinate, to generate tetrahydrodipicolinate. This enzyme catalyzes the second step in the bacterial biosynthetic pathway that generates meso-diaminopimelate, a component of bacterial cell walls, and the amino acid
L-lysine. The *Mycobacterium tuberculosis* dapB-encoded DHPR has been cloned, expressed, purified, and crystallized in two ternary complexes with NADH or NADPH and the inhibitor 2,6-pyridinedicarboxylate (2,6-PDC). The structures have been solved using molecular replacement strategies, and the DHPR-NADH-2,6-PDC and DHPR-NADPH-2,6-PDC complexes have been refined against data to 2.3 and 2.5 Å, respectively. The *M. tuberculosis* DHPR is a tetramer of identical subunits, with each subunit composed of two domains connected by two flexible hinge regions. The N-terminal domain binds pyridine nucleotide, while the C-terminal domain is involved in both tetramer formation and substrate/inhibitor binding. The *M. tuberculosis* DHPR uses NADH and NADPH with nearly equal efficiency based on V/K values. To probe the nature of this substrate specificity, we have generated two mutants, K9A and K11A, residues that are close to the 2'-phosphate of NADPH. These two mutants exhibit decreased specificity for NADPH by factors of 6- and 30-fold, respectively, but the K11A mutant exhibits 270% of WT activity using NADH. The highly conserved structure of the nucleotide fold may permit other enzyme’s nucleotide specificity to be altered using similar mutagenic strategies.—Authors’ Abstract


Pathogenic mycobacteria, including the causative agents of tuberculosis and leprosy, are responsible for considerable morbidity and mortality worldwide. A hallmark of these pathogens is their tendency to establish chronic infections that produce similar pathologies in a variety of hosts. During infection, mycobacteria reside in macrophages and induce the formation of granulomas, organized immune complexes of differentiated macrophages, lymphocytes, and other cells. This review summarizes our understanding of Mycobacterium-host cell interactions, the bacterial-granuloma interface, and mechanisms of bacterial virulence and persistence. In addition, we highlight current controversies and unanswered questions in these areas.—Authors’ Abstract


Although IFN-gamma is necessary for survival of *Mycobacterium tuberculosis* infection in people and animal models, it may not be sufficient to clear the infection, and IFN-gamma is not a reliable correlate of protection. To determine whether IFN-gamma-independent mechanisms of immunity exist, we developed a murine *ex vivo* culture system that directly evaluates the ability of splenic or lung lymphocytes to control the growth of *M. tuberculosis* within infected macrophages, and that models *in vivo* immunity to tuberculosis. Surprisingly, CD4(+) T cells controlled >90% of intracellular *M. tuberculosis* growth in the complete absence of IFN-gamma stimulation of macrophages, via a NO-dependent mechanism. Furthermore, bacillus Calmette-Guerin-vaccinated IFN-gamma-deficient mice exhibited significant protection against *M. tuberculosis* challenge that was lost upon depletion of CD4(+) T cells. These findings demonstrate that CD4(+) T cells possess IFN-gamma-independent mechanisms that can limit the growth of an intracellular pathogen and are dominant in secondary responses to *M. tuberculosis*.—Authors’ Abstract


*Mycobacterium tuberculosis*, the causative agent of tuberculosis, possesses a class I b ribonucleotide reductase (RNR), encoded by the *nrdE* and *nrdF2* genes, in ad-
dition to a putative class II RNR, encoded by nrdZ. In this study we probed the relative contributions of these RNRs to the growth and persistence of *M. tuberculosis*. We found that targeted knockout of the nrdF2 gene could be achieved only in the presence of a complementing allele, confirming that this gene is essential under normal, in vitro growth conditions. This observation also implied that the alternate class Ib small subunit encoded by the nrdF1 gene is unable to substitute for nrdF2 and that the class II RNR, NrdZ, cannot substitute for the class Ib enzyme, NrdEF2. Conversely, a DeltanrdZ null mutant of *M. tuberculosis* was readily obtained by allelic exchange mutagenesis. Quantification of levels of nrdE, nrdF2, nrdF1, and nrdZ gene expression by real-time, quantitative reverse transcription-PCR with molecular beacons by using mRNA from aerobic and O(2)-limited cultures showed that nrdZ was significantly induced under microaerophilic conditions, in contrast to the other genes, whose expression was reduced by O(2) restriction. However, survival of the DeltanrdZ mutant strain was not impaired under hypoxic conditions in vitro. Moreover, the lungs of B6D2/F(1) mice infected with the DeltanrdZ mutant had bacterial loads comparable to those of lungs infected with the parental wild-type strain, which argues against the hypothesis that nrdZ plays a significant role in the virulence of *M. tuberculosis* in this mouse model.—Author’s Abstract


Studies in mouse infection models clearly demonstrate tumour necrosis factor (TNF) to be a critical component of both the antibacterially protective and the inflammatory immune response to *Mycobacterium tuberculosis*. It is therefore not surprising that treatment of patients—for example, those with rheumatoid arthritis—with biological agents interfering with TNF activity have shown an increased risk of reactivating tuberculosis. However, conceivably, TNF targeting biological agents can be developed that because of their particular mode of action and their specific pharmacodynamics may be less likely to have this side effect.—Author’s Abstract


Up-regulation of expression of the cell-surface marker CD44 is a major characteristic of T lymphocytes responding in the lungs of mice infected with *Mycobacterium tuberculosis*. These lymphocytes express an activated/memory phenotype as seen by their high expression of the CD44 molecule and low expression of CD62L and CD45RB cell-surface molecules. Based on increasing evidence that the CD44 molecule participates in several aspects of the inflammatory response, we evaluated its role in the response to infection with *M. tuberculosis* using gene-disrupted mice. In this report, we show that CD44 expression is not necessary for the proper trafficking of protective cells to the lungs of mice infected with *M. tuberculosis* or the direct expression of protective immunity leading to control and containment of the bacterial load in this organ. However, although there were no differences in the bacterial load or migration of activated T lymphocytes to the inflamed lung, the absence of the CD44 molecule resulted in a substantially increased accumulation of neutrophils in the lung. These data indicate that loss of CD44 expression does not alter expression of T helper cell type 1 immunity to tuberculosis in the lungs but has major effects on the overall cellular composition of the immunopathological response.—Authors’ Abstract

In humans and in mice, control of the intracellular pathogen, Mycobacterium tuberculosis (Mtcb), requires IFN-gamma. Although the adaptive immune response results in production of substantial amounts of IFN-gamma in response to Mtcb, the immune response is unable to eradicate the infection in most cases. We have previously reported evidence that Mtcb inhibits macrophage responses to IFN-gamma, suggesting that this may limit the ability of IFN-gamma to stimulate macrophages to kill Mtcb. We have also observed that uninfected macrophages, adjacent to infected macrophages in culture, exhibit decreased responses to IFN-gamma. Here we report that IL-6 secreted by Mtcb-infected macrophages inhibits the responses of uninfected macrophages to IFN-gamma. IL-6 selectively inhibits a subset of IFN-gamma-responsive genes at the level of transcriptional activation without inhibiting activation or function of STAT1. Inhibition of macrophage responses to IFN-gamma by IL-6 requires new protein synthesis, but this effect is not attributable to suppressor of cytokine signaling 1 or 3. These results reveal a novel function for IL-6 and indicate that IL-6 secreted by Mtcb-infected macrophages may contribute to the inability of the cellular immune response to eradicate infection.—Authors’ Abstract


Proteins encoded by DNA segment RD1 of Mycobacterium tuberculosis have recently been demonstrated to play important roles in bacterial virulence, vaccine development, and diagnostic reagent design. Previously, we characterized two immunodominant T-cell antigens, the early secreted antigen target (ESAT-6) and the 10-kDa culture filtrate protein (CFP10), which are encoded by the esx-lhp operon in this region. In the present study we characterized a third putative open reading frame in this region, rv3873, which encodes a PPE protein. We found that the rv3873 gene is expressed in M. tuberculosis H37Rv and that the native protein, Rv3873, is predominantly associated with the mycobacterial cell or wall. When tested as a His-tagged recombinant protein, Rv3873 stimulated high levels of gamma interferon secretion in peripheral blood mononuclear cells isolated from tuberculosis (TB) patients, as well as from healthy tuberculin purified protein derivative-positive donors. In contrast to other RD1-encoded antigens, Rv3873 was also found to be recognized by a significant proportion of Mycobacterium bovis BCG-vaccinated donors. Epitope mapping performed with overlapping peptides revealed a broad pattern of T-cell recognition comprising both TB-specific epitopes and epitopes also recognized by BCG-vaccinated donors. The immunodominant epitope (residues 118 to 135) for both TB patients and BCG-vaccinated individuals was found to be highly conserved among a large number of PPE family members.—Authors’ Abstract


Among the first cells to invade a site of infection, polymorphonuclear neutrophils (PMN) play an important role in the control of numerous infections. While PMN are considered critical for control of acute infections, their role in chronic infections remains less well understood. Here we report that PMN are essential for accurate early granuloma formation during chronic M. tuberculosis infection without influencing mycobacterial growth restriction. The PMN-mediated regulation of granuloma formation depended on chemokines signaling through CXCR3, in particular MIG, as indicated by immune histochemical analysis of lung sections from C57BL/6 wild-type and CXCR3(−/−) mutant mice and sup-
ported by microarray transcriptome analysis. Hence, PMN play a central role in regulating the focal granulomatous response in the lung, and this early granuloma formation can be segregated from long-term protection against pulmonary *M. tuberculosis* infection.—Authors’ Abstract


*Mycobacterium tuberculosis* antigens that are recognized by human CD8+ T cells are potentially important vaccine target molecules. We used a motif-based strategy to screen selected proteins of *M. tuberculosis* for peptides predicted to bind to human leukocyte antigen (HLA)-A*0201. We identified two 10 amino acid peptides that elicited cytolysis of T lymphocyte activity and interferon-gamma production by CD8+ T cells from HLA-A*0201+ healthy tuberculin reactors. These peptides were derived from the 38-kDa antigen and the 28-kDa hemolysin, the latter being a novel target for CD8+ T cells. We speculate that hemolysins may alter the phagosomal membrane surrounding intracellular *M. tuberculosis*, allowing themselves and other antigens to gain access to the major histocompatibility complex class I processing pathway.—Authors’ Abstract


Macrophages are activated from a resting state by a combination of cytokines and microbial products. Microbes are often sensed through Toll-like receptors signaling through MyD88. We used large-scale microarrays in multiple replicate experiments followed by stringent statistical analysis to compare gene expression in wild-type (WT) and MyD88−/− macrophages. We confirmed key results by quantitative reverse transcription polymerase chain reaction, Western blot, and enzyme-linked immunosorbent assay. Surprisingly, many genes, such as inducible nitric oxide synthase, IRG-1, IP-10, MIG, RANTES, and interleukin 6 were induced by interferon (IFN)-gamma from 5- to 100-fold less extensively in MyD88−/− macrophages than in WT macrophages. Thus, widespread, full-scale activation of macrophages by IFN-gamma requires MyD88. Analysis of the mechanism revealed that MyD88 mediates a process of self-priming by which resting macrophages produce a low level of tumor necrosis factor. This and other factors lead to basal activation of nuclear factor kappaB, which synergizes with IFN-gamma for gene induction. In contrast, infection by live, virulent *Mycobacterium tuberculosis* (Mt) activated macrophages largely through MyD88-independent pathways, and macrophages did not need MyD88 to kill Mt in vitro. Thus, MyD88 plays a dynamic role in resting macrophages that supports IFN-gamma-dependent activation, whereas macrophages can respond to a complex microbial stimulus, the tubercle bacillus, chiefly by other routes.—Authors’ Abstract


We investigated the effectiveness of supportive therapy with a fish-oil extract called repair tuberculosis (RTB) in anti-tuberculosis treatment, and the underlying mechanism of action. The active component of RTB is the unsaturated fatty acid docosatetraenoic acid (C(22)H(36)O(2)), which was reported to induce the resorption and healing of pulmonary lesions in patients with severe pulmonary tuberculosis. We administered RTB to a rat model of CFA-induced pulmonary tuberculous granuloma (RTB group), and
compared the results with those in a control group, which did not receive RTB. Histological examination of the lungs showed a significantly smaller area of granuloma in the RTB group than in the control group. IFN-gamma levels in bronchoalveolar lavage fluid (BALF) were higher in the RTB group than in the control group, suggesting that Th1-type immune reaction is activated in the RTB group. Moreover, significantly enhanced expression of inducible nitric oxide synthase mRNA in lung tissue was observed in the RTB group. Superoxide production by cells recovered from BALF was attenuated in the RTB group. There were no difference in IL-4 levels in BALF, or in expression of TNF-alpha mRNA in lung tissue between the RTB and control groups. The above results suggest that RTB activates Th1-type cellular immune reaction, promotes absorption of lesions, and inhibits the generation of cytotoxic substances.—Authors’ Abstract


Although Mycobacterium marinum is closely related to Mycobacterium tuberculosis H37Rv genomically, the clinical outcome in humans is quite different for M. marinum and M. tuberculosis infections. We investigated possible factors in the host macrophages for determining differential pathological responses to M. tuberculosis and M. marinum using an in vitro model of mycobacterial infection. Using suppression-subtractive hybridization, we identified 12 differentially expressed genes in the human monocytic cell line U937 infected with M. tuberculosis and M. marinum. Of those genes, the most frequently recovered transcript encoded interleukin-8 (IL-8). Northern hybridization revealed that IL-8 mRNA was highly upregulated in M. tuberculosis-infected U937 cells compared with M. marinum-infected cells. In addition, enzyme-linked immunosorbent assay showed that IL-8 protein secretion was significantly elevated in M. tuberculosis-infected U937 cells, human primary monocytes, and monocyte-derived macrophages compared with that in M. marinum-infected cells. The depressed IL-8 expression was unique in M. marinum-infected cells compared with cells infected with other strains of mycobacteria, including M. tuberculosis H37Ra, Mycobacterium bovis BCG, or Mycobacterium smegmatis. When the expression of NF-kappaB was assessed in mycobacterium-infected U937 cells, IkkappaBalpha proteins were significantly degraded in M. tuberculosis-infected cells compared with M. marinum-infected cells. Collectively,
these results suggest that differential IL-8 expression in human macrophages infected with *M. tuberculosis* and *M. marinum* may be critically associated with distinct host responses in tuberculosis. Additionally, our data indicate that differential signal transduction pathways may underlie the distinct patterns of IL-8 secretion in cells infected by the two mycobacteria.—Authors’ Abstract

### Microbiology

**Alexander, D. C., Jones, J. R., and Liu, J.**


Rifampin is a front-line antibiotic for the treatment of tuberculosis. Infections caused by rifampin- and multidrug-resistant *Mycobacterium tuberculosis* strains are difficult to treat and contribute to a poor clinical outcome. Rifampin resistance most often results from mutations in rpoB. However, some drug-resistant strains have rpoB alleles that encode the phenotype for susceptibility. Similarly, non-*M. tuberculosis* mycobacteria exhibit higher levels of baseline resistance to rifampin, despite the presence of rpoB alleles that encode the phenotype for susceptibility. To identify other genes involved in rifampin resistance, we generated a library of *Mycobacterium smegmatis* mc(2)155 transposon insertion mutants. Upon screening this library, we identified one mutant that was hypersensitive to rifampin. The transposon insertion was localized to the arr gene, which encodes rifampin ADP ribosyltransferase, an enzyme able to inactivate rifampin. Sequence analysis revealed differences in the arr alleles of *M. smegmatis* strain mc(2)155 and previously described strain DSM 43756. The arr region of strain mc(2)155 contains a second, partial copy of the arr gene plus a novel insertion sequence, IS1623.—Authors’ Abstract


Sensitive and specific techniques to detect and identify *Mycobacterium tuberculosis* directly in clinical specimens are important for the diagnosis and management of patients with tuberculosis (TB). We developed two real-time PCR assays, based on the IS6110 multicopy element and on the senX3-regX3 intergenic region, which provide a rapid method for the diagnosis of mycobacterial infections. The sensitivity and specificity of both assays were established by using purified DNA from 71 clinical isolates and 121 clinical samples collected from 83 patients, 20 of whom were affected by TB. Both assays are accurate, sensitive, and specific, showing a complementary pattern of Mycobacterium recognition: broader for the IS6110-based assay and restricted to the *M. tuberculosis* complex for the senX3-regX3-based assay. Moreover, the addition of a synthetic DNA calibrator prior to DNA extraction allowed us to measure the efficiency of DNA recovery and to control for the presence of PCR inhibitors. The mycobacterial burden of the clinical samples, as assessed by direct microscopy, correlates with the *M. tuberculosis* DNA load measured by the senX3-regX3-based assay. In addition, reduced levels of *M. tuberculosis* DNA load are present in those patients subjected to successful therapy, suggesting a potential use of this assay for monitoring treatment efficacy. Therefore, these assays represent a fully controlled high-throughput system for the evaluation of mycobacterial burden in clinical specimens.—Authors’ Abstract

Sequencing of the 16S ribosomal DNA (rDNA) for identification of nontuberculous mycobacteria (NTM) has contributed to the establishment of more than 35 new species during the last decade. Increasingly, NTM are accepted as potential or proven pathogens. We identified, by 16S rDNA sequence analysis, slowly growing NTM isolates negative by AccuProbe (GenProbe, San Diego, CA) that previously were identified by using conventional biochemical techniques, to determine the accuracy of reporting AccuProbe-negative NTM prior to sequence-based identification. Of 82 strains, 30 were deemed novel. An attempt was made to determine the clinical importance of previously misidentified novel species. Clinical cases are described for a number of strains previously identified as Mycobacterium terrae complex, Mycobacterium scrofulaceum, and Mycobacterium avium complex. As sequence-based identification methods become more commonplace in clinical microbiology laboratories, there is a need to understand the significance of previously undescribed species, which often mimic and subsequently are identified as well-established species.—Authors’ Abstract


OBJECTIVE: To observe the resistance of Mycobacterium abscessus to rifampin and to investigate if there is any mutation of rpoB gene in strains with high minimal inhibitory concentration (MIC). METHODS: Mycobacterium abscessus was identified with both biochemical methods and PCR-RFLP. The MICs of rifampin to all the clinical strains and the type strain ATCC19977 were determined. DNA sequences were obtained from a 1272 bp fragment of the rpoB gene from either low or high rifampin MIC strains using PCR amplification. RESULTS: Only one strain had low MICs of 4 micro g/ml. MICs of all the other 14 strains, as well as the type strain were above 128–256 micro g/ml. Although there were some differences in nucleotide sequence of rpoB gene, all strains had the same amino acid sequence without any mutations. CONCLUSIONS: Mycobacterium abscessus showed a high resistance to rifampin. Mutations of rpoB gene do not seem to be the responsible mechanism.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 115.


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See Current Literature, Molecular and Genetic Studies, p. 115.
Microbiology (Leprosy)


We report the use of a single-tube homogenization/RNA extraction method that produces enough RNA to study the expression of 30 genes from a single skin biopsy specimen of a multibacillary leprosy patient and demonstrate that RNA can be purified after fixation of biopsies in 70% ethanol for up to a year. This represents a major advancement in the ability to study M. leprae gene expression directly from biopsy material and should help to define genes that are associated with intracellular survival of this human pathogen.—Authors’ Abstract

Microbiology (Tuberculosis)


Tuberculosis (TB) is a major concern in developing countries. In Brazil, few genotyping studies have been conducted to verify the number of IS6110 copies present in local prevalent strains of Mycobacterium tuberculosis, the distribution and clustering of strains. IS6110 DNA fingerprinting was performed on a sample of M. tuberculosis isolates from patients with smear-positive pulmonary TB, at a hospital in Brazil. The IS6110 profiles were analyzed and compared to a M. tuberculosis database of the Houston Tuberculosis Initiative, Houston, US. Seventy-six fingerprints were obtained from 98 patients. All M. tuberculosis strains had an IS6110 copy number between 5–21 allowing for differentiation of the isolates. Human immunodeficiency virus infection was confirmed in nearly half the patients of whom data was available. Fifty-eight strains had unique patterns, while 17 strains were grouped in 7 clusters (2 to 6 strains). When compared to the HTI database, 6 strains matched isolates from El Paso, Ciudad de Juarez, Houston, and New York. Recently acquired infections were documented in 19% of cases. The community transmission of infection is intense, since some clustered strains were recovered during the four-year study period. The intercontinental dissemination of M. tuberculosis strains is suspected by demonstration of identical fingerprints in a distant country.—Authors’ Abstract


Tuberculosis (TB) has afflicted humankind throughout history. Approximately one third of the world’s population is currently infected with Mycobacterium tuberculosis and nearly two million people die of TB annually. Although much has been learned about the structure of the tubercle bacillus, the epidemiology of TB, the physiological and immunological responses of the host to infection, and the physiology of M. tuberculosis in laboratory broth cultures, much of the basic biology of M. tuberculo-
sis in its natural setting (the infected human) remains to be elucidated. Within the past decade, there have been remarkable advances in the development of genetic and molecular biological tools with which to study M. tuberculosis. This review discusses the approaches that have been employed and the progress that has been made in discovering how M. tuberculosis has achieved its prowess as a successful pathogen.—Authors’ Abstract


The recA gene of Mycobacterium tuberculosis is unusual in that it is expressed from two promoters, one of which, P1, is DNA damage inducible independently of LexA and RecA, while the other, P2, is regulated by LexA in the classical way (E. O. Davis, B. Springer, K. K. Gopaul, K. G. Papavinasasundaram, P. Sander, and E. C. Bottger, Mol. Microbiol. 46:791–800, 2002). In this study we characterized these two promoters in more detail. Firstly, we localized the promoter elements for each of the promoters, and in so doing we identified a mutation in each promoter which eliminates promoter activity. Interestingly, a motif with similarity to Escherichia coli sigma(70) –35 elements but located much closer to the –10 element is important for optimal expression of P1, whereas the sequence at the –35 location is not. Secondly, we found that the sequences flanking the promoters can have a profound effect on the expression level directed by each of the promoters. Finally, we examined the contribution of each of the promoters to recA expression and compared their kinetics of induction following DNA damage.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 111.


Mycobacterium tuberculosis is an infectious microorganism that causes human tuberculosis. The cell membranes of pathogens are known to be rich in possible diagnostic and therapeutic protein targets. To complement the M. tuberculosis genome, we have profiled the membrane protein fraction of the M. tuberculosis H37Rv strain using an analytical platform that couples one-dimensional SDS gels to a microcapillary liquid chromatography-nanospray-tandem mass spectrometer. As a result, 739 proteins have been identified by two or more distinct peptide sequences and have been characterized. Interestingly, approximately 450 proteins represent novel identifications, 79 of which are membrane proteins and more than 100 of which are membrane-associated proteins. The physicochemical properties of the identified proteins were studied in detail, and then biological functions were obtained by sorting them according to Sanger Institute gene function category. Many membrane proteins were found to be involved in the cell envelope, and those proteins with energy metabolic functions were also identified in this study.—Authors’ Abstract


Studying defined mutants of Mycobacterium tuberculosis in the mouse model of infection has led to the discovery of attenu-
ated mutants that fall into several phenotypic classes. These mutants are categorized by their growth characteristics compared with those of wild-type *M. tuberculosis*, and include severe growth *in vivo* mutants, *growth in vivo* mutants, persistence mutants, pathology mutants and dissemination mutants. Here, examples of each of these mutant phenotypes are described and classified accordingly. Defining the importance of mycobacterial gene products responsible for *in vivo* growth, persistence and the induction of immunopathology will lead to a greater understanding of the host-pathogen interaction and potentially to new antmycobacterial treatment options.—Authors’ Abstract


The sequencing of the complete genome of *M. tuberculosis* H37Rv has resulted in the recognition of four mce operons in its genome by in silico analysis. In an attempt to understand the significance of the redundancy of mce operons, we analyzed the expression profile of mce operons after different periods of growth in culture as well as during *in vivo* infection. Our results strongly suggest that mce1 is expressed as a polycistronic message. In culture from day 8 to day 12, expression of only mce1 was observed, but as the cultures progress towards stationary phase the expression profile of mce operons was altered; the transcripts of the mce1 operon were barely detected while those of the mce4 operon were prominent. In an analysis of the expression of mce operons in tubercle material collected from infected animal tissues, we detected the expression of mce1, -3 and -4. Our results imply that mce operons other than mce1 are also expressed during infection and that it is necessary to examine their role in pathogenesis.—Authors’ Abstract


Both CD4+ and CD8+ T cells are important for successful immunity to tuberculosis and have redundant effector functions, such as cytolysis and release of potent antmycobacterial cytokines such as interferon-gamma and tumor necrosis factor-alpha. We hypothesized that CD8+ T cells play a unique role in host defense to *Mycobacterium tuberculosis* infection as well. Possibilities include preferential and/or enhanced release of granular constituents and/or preferential recognition of heavily infected cells. Utilizing human, *Mycobacterium tuberculosis*-specific, CD4+ and CD8+ T cell clones, we demonstrate that, after recognition of antigen-presenting cells displaying peptide antigen, CD4+ T cells preferentially release interferon-gamma, whereas CD8+ T cells preferentially lyse antigen-presenting cells. Furthermore, utilizing dendritic cells infected with *Mycobacterium tuberculosis* expressing green fluorescent protein, we show that CD8+ T cells preferentially recognize heavily infected cells that constitute the minority of infected cells. These data support the hypothesis that the central role of CD8+ T cells in the control of infection with *Mycobacterium tuberculosis* may be that of surveillance; in essence, recognition of cells in which the containment of *Mycobacterium tuberculosis* is no longer effective.—Authors’ Abstract


Two hundred three freeze-dried strains of *Mycobacterium tuberculosis* collected during the 1960s were compared with 4102 strains collected during the 1990s, and 14 DNA patterns identified among the “historical strains” were 100% identical to patterns identified among the “recent strains.” They were isolated from 41 and 40 patients who
had tuberculosis during the 1960s and 1990s, respectively. The patients’ mean age differed by >30 years, a finding strongly suggesting that the patients from the 1990s experienced reactivation of \textit{M. tuberculosis} infection acquired during the 1960s. The half-life of IS6110 DNA patterns during latency was estimated to be 36 years (95% confidence interval, 25–54 years). Thus, this comparison of historical and recent strains yields molecular epidemiologic evidence of \textit{M. tuberculosis} reactivation spanning decades and suggests that the rate of change of DNA patterns during latency is much longer than that during active disease. This has important implications for the interpretation of clustering, especially for the extent of recent transmission.—Authors’ Abstract


The emergence of multidrug-resistant tuberculosis calls for new, rapid drug susceptibility tests. We have tested 150 \textit{Mycobacterium tuberculosis} isolates against the second-line drugs ethionamide, kanamycin, capreomycin, ofloxacin, and paraaminosalicylic acid by the colorimetric resazurin microtiter assay and the proportion method. By visual reading, MICs were obtained after 8 days. A very good correlation between results by the colorimetric resazurin microtiter assay and the proportion method was obtained. The colorimetric resazurin microtiter assay is inexpensive, rapid, and simple to perform, and implementation of the assay is feasible for low-resource countries.—Authors’ Abstract


Ziehl-Neelsen (ZN) staining is the key technique for diagnosis of mycobacterial infections; however, a high percentage of patients exhibit positive signs of tuberculosis, as indicated by pathology, culture of mycobacteria, and polymerase chain-reaction analysis, and yet show negative results on ZN staining. In this report we present evidence that such ZN-negative specimens represent \textit{Mycobacterium tuberculosis} bacilli in a dormant state with distinct cell-wall alterations: the classical cell-wall composition-dependent ZN staining of \textit{M. tuberculosis} in lung sections gradually discontinued with persistence of infection, both in mice and in human patients; in contrast, detection of mycobacteria by cell-wall composition-independent staining using a polyclonal anti-\textit{M. bovis} Bacille-Calmette-Guerin serum continued with persistence of infection. These findings have important implications for diagnosis, as well as for both chemotherapy and development of vaccine strategies.’—Authors’ Abstract


The amplified-fragment length polymorphism (AFLP) technique was applied to clusters of \textit{Mycobacterium tuberculosis} clinical isolates obtained by using IS6110-based restriction fragment length polymorphism (RFLP). Ten of the RFLP clusters showed identical AFLP patterns also, but the other 13 could be resolved into subclusters by AFLP. Our results suggest that some RFLP clusters may not be due to recent transmission and that AFLP may be a useful complementary technique.—Authors’ Abstract

Probing protein extracts from exponentially growing and stationary phase cultures of Mycobacterium bovis BCG with anti-phospho amino acid antibodies revealed a 31-kDa anti-phospho threonine antibody-reactive protein specific to growing culture. The corresponding protein was purified via two-dimensional gel electrophoresis and identified via mass spectrometry to be malonyl coenzyme A:acyl carrier protein transacylase (MCAT), a component of the fatty acid biosynthetic pathway. MCAT tagged with histidine reacted with anti-phospho threonine antibody and was positive in an in-gel chemical assay for phospho proteins. Analysis of the growth phase dependence of MCAT-His phosphorylation and protein levels showed that phosphorylated MCAT-His can be detected only in growing culture. In contrast, MCAT-His protein level was growth phase-independent. These results suggest that MCAT may be a substrate of a protein kinase and phosphatase, and that aspects of fatty acid synthesis in tubercle bacilli are regulated by protein phosphorylation.—Authors’ Abstract


Although many bacterial pathogens use specialized secretion systems for virulence, no such systems have been described for Mycobacterium tuberculosis, a major pathogen of humans that proliferates in host macrophages. In a screen to identify genes required for virulence of M. tuberculosis, we have discovered three components and two substrates of the first Sec-independent secretion pathway described in M. tuberculosis, which we designate the Snm pathway. Here we demonstrate that the proteins Snm1, -2, and -4 are required for the secretion of ESAT-6 and CFP-10, small proteins previously identified as major T cell antigens. Snm2, a member of the AAA ATPase family, interacts with substrates and with Snm1, another AAA ATPase. We show that M. tuberculosis mutants lacking either the Snm system or these substrates exhibit defects in bacterial growth during the acute phase of a mouse infection and are attenuated for virulence. Strikingly, snm mutants fail to replicate in cultured macrophages and to inhibit macrophage inflammatory responses, two well established activities of wild-type M. tuberculosis bacilli. Thus, the Snm secretion pathway works to subvert normal macrophage responses and is a major determinant of M. tuberculosis virulence.—Authors’ Abstract


Tuberculosis remains one of the leading infectious causes of death worldwide. The emergence of drug-resistant strains of Mycobacterium tuberculosis is a serious public health threat. Resistance to isoniazid (INH) is the most prevalent form of resistance in M. tuberculosis and is mainly caused by mutations in the catalase peroxidase gene (katG). Among high-level INH-resistant isolates (MIC > or = 2), 89% are associated with a mutation at codon 315 of katG. There is a need to develop rapid diagnostic tests to permit appropriate antibiotic treatment and to improve clinical management. Therefore, a single-tube real-time PCR, using a novel kind of probe (3′-minor groove binder-DNA probe), was developed to detect either the wild-type or the mutant codon directly in Ziehl-Neelsen-positive sputum samples. The detection limit of the assay for purified DNA was 5 fg per well (one mycobacterial genome), and with spiked sputum samples, it was 20 copies per well, corresponding to 10(3) mycobacteria per ml of sputum. Sputum samples from 20 patients living in Kazakhstan or Moldova and infected with monodrug- or multidrug-resistant M. tuberculosis and 20 sputum samples from patients infected with INH-susceptible M. tuberculosis were tested. The sensitivities and specificities of
the probes were 70 and 94% for the wild-type probe and 82 and 100% for the mutant probe. Binding to either probe was nonambiguous. This real-time PCR allows the rapid identification of a mutant katG allele and can easily be implemented in a clinical microbiology laboratory.—Authors’ Abstract


A novel polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis (PRA) of the hsp65 gene was used for the routine identification of mycobacteria in a high throughput clinical laboratory. A total of 2036 clinical isolates were tested by PRA in conjunction with other methods. The PRA identification of _M. tuberculosis_ complex was 100% sensitive and specific, and 74.5% of nontuberculous mycobacteria (NTM) were correctly identified. It gave highly consistent results for _Mycobacterium avium_ complex (MAC) species and for most isolates of _M. fortuitum, M. chelonae_, and _M. kansasii_. It had proven to be highly robust and stable despite usage on such a large-scale and is thus particularly suitable for use in high throughput laboratories in areas with a high incidence of tuberculosis.—Authors’ Abstract

__Experimental Infections__


Nonhuman primates were used to develop an animal model that closely mimics human _Mycobacterium tuberculosis_ infection. Cynomolgus macaques were infected with low doses of virulent _M. tuberculosis_ via bronchoscopic instillation into the lung. All monkeys were successfully infected, based on tuberculin skin test conversion and peripheral immune responses to _M. tuberculosis_ antigens. Progression of infection in the 17 monkeys studied was variable. Active-chronic infection, observed in 50 to 60% of monkeys, was characterized by clear signs of infection or disease on serial thoracic radiographs and in other tests and was typified by eventual progression to advanced disease. Approximately 40% of monkeys did not progress to disease in the 15 to 20 months of study, although they were clearly infected initially. These monkeys had clinical characteristics of latent tuberculosis in humans. Low-dose infection of cynomolgus macaques appears to represent the full spectrum of human _M. tuberculosis_ infection and will be an excellent model for the study of pathogenesis and immunology of this infection. In addition, this model will provide an opportunity to study the latent _M. tuberculosis_ infection observed in approximately 90% of all infected humans.—Authors’ Abstract


Inbred strains of mice exhibit varied patterns of susceptibility following infection with virulent _Mycobacterium tuberculosis_. Susceptible mice have progressive fulminate disease resulting in their premature death; in contrast, resistant mice are able to control bacterial replication, limit lung injury and survive longer. The use of these mouse strains in experimental infection has allowed the identification of immunological correlates of protective versus unsuccessful
host responses to tuberculosis, and the identification of susceptibility loci by combining classical and molecular genetics. These immunological and genetic features that distinguish susceptible and resistant inbred mouse strains may allow us to better understand susceptibility to tuberculous disease in people. A possible benefit would be the delineation of markers of protective immunity for use in vaccine development. This is a review of recent insights into the genetics and immunology of resistance and susceptibility to virulent *M. tuberculosis* using genetically intact mice.—Authors’ Abstract


L-Lysine HCl is being proposed to be a possible biocompatible adjuvant to enhance immune response by virtue of its probable non-specific bridging action and cellular proliferation properties. This proposal has been tried to be substantiated by carrying out experimentation where L-lysine HCl has been used as an adjuvant (various groups based on mode of application and frequency of booster dose were designed) in tuberculosis vaccination experiments with heat killed *Mycobacterium tuberculosis* (MTB) and Bacille Calmette Guerin (BCG). Antibody titre has been followed in all the experiments as a measure of immune response. Amongst the various groups designed, group 1A (L-lysine HCl was given at a separate site as that of the antigen; lysine booster was given to this group intermittently, i.e. lysine given on 0th, 7th, 14th, 21st days of immunization) came out as the stand-alone leader. This mode and frequency of application was then compared with a group which received a standard adjuvant, viz. alhydrogel. Results were obtained which showed the following order in terms of decreasing antibody titre: alhydrogel group > lysine group > control group. Considering the biocompatible nature of lysine in comparison with the reportedly hazardous nature of alum adjuvants, we propose L-lysine HCl as a probable adjuvant in vaccination.—Authors’ Abstract


Cell-mediated immunity is considered to be the major component of the host response against *Mycobacterium tuberculosis*, whereas antibody-mediated immunity historically has been considered inconsequential. In recent years, studies from several groups have challenged the traditional dogma and demonstrated that monoclonal antibodies can modify various aspects of mycobacterial infections. This review describes the experimental evidence supporting a role for antibodies in defense against mycobacterial infections and outlines future challenges to the field of antibody-mediated immunity against *M. tuberculosis*, with particular emphasis on the implications of these findings for a novel vaccine strategy. —Author’s Abstract


The rabbit model of tuberculosis has been used historically to differentiate between *Mycobacterium tuberculosis* and *Mycobacterium bovis* based on their relative virulence in this animal host. *M. tuberculosis* infection in market rabbits is cleared over time, whereas infection with *M. bovis* results in chronic, progressive, cavitary disease leading to death. Because of the innate resistance of commercial rabbits to *M. tuberculosis*, 320 to 1,890 log-phase, actively growing inhaled bacilli were required to form one grossly visible pulmonary tubercle at 5 weeks. The range of inhaled doses required to make one tubercle allows us to determine the relative pathogenicities of different strains. Fewer inhaled doses required to make one tubercle allows us to determine the relative pathogenicities of different strains. Fewer inhaled organisms of the *M. tuberculosis* Erdman strain were required than of *M. tuberculosis* H37Rv to produce a visible lesion at 5 weeks. Fur-
thermore, with the Erdman strain, only 7 of 15 rabbits had healed lesions at 16 to 18 weeks; among the other animals, two had chronic, progressive cavitary disease, a phenotype usually seen only with *M. bovis* infection. Genotypic investigation of the Erdman strain with an H37Rv-based microarray identified gene differences in the RD6 region. Southern blot and PCR structural genetic analysis showed significant differences between *M. tuberculosis* strains in this region. Correlation of the relative pathogenicity, including disease severity, in the rabbit model with the strain genotype may help identify stage-specific *M. tuberculosis* genes important in human disease. —Authors’ Abstract


The widely administered *Mycobacterium bovis* BCG is an attractive live vector for the development of AIDS vaccines. We explored immune responses induced in cynomolgus macaques to rBCG-SIV(3), a mixture of three recombinant BCG strains expressing the SIVmac251 nef, gag and env genes. After a single intradermal (ID) inoculation, circulating blood cells from rBCG-SIV(3)-vaccinated monkeys exhibited CTL responses targeted against the three antigens and interferon-gamma (IFNgamma) secretion was observed. A rectal or oral boosting dose of rBCG-SIV(3) elicited anti-SIV IgAs in the rectum of vaccinated monkeys and increased IFNgamma secretion by circulating blood cells. Despite a good response against the vector, rBCG-SIV(3) administration did not induce IgG antibody responses or lymphoproliferation against the SIV antigens in blood. This could be due to the lack of in vivo persistence of the recombinant BCG strains that were used. Rectal challenge with fully pathogenic SIVmac251-infected all animals. However, after viral challenge, anti-SIV cellular and antibody responses were higher in rBCG-SIV(3) monkeys than in controls indicating that the vaccine induced anti-SIV CD4(+) T-cell memory.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 112.


The widely used vaccine against tuberculosis, BCG, shows evidence of genetic instability. It has undergone major genetic rearrangements resulting in deletion and duplication of segments of its chromosome. In order to produce a BCG strain with more favorable genetic properties, we inactivated the recA gene. Targeted deletion of the recA gene of BCG resulted in a complete loss of recombination between homologous, chromosomally-located sequences, as well as between plasmid- and chromosomally-located sequences. The deltarecA mutant BCG was as effective as the wild-type in conferring protection in mice against an intravenous challenge with virulent *Mycobacterium tuberculosis*, indicating that the loss of an SOS response-mediated DNA repair mechanism did not compromise the immunological properties of BCG. The availability of a genetically stable, fully immunogenic BCG is important for the future development of BCG as a live vaccine.—Authors’ Abstract

**Santucci, M. B., Ciaramella, A., Mattei, M., Sumerska, T., and Fraziano, M.** Batimastat reduces *Mycobacterium tuberculosis*-induced apoptosis in macro-

In this study, we report evidences that Mycobacterium tuberculosis (MTB)-induced apoptosis in macrophages is reduced by a broad-spectrum hydroxamic acid-based matrix metalloproteinase (MMP) inhibitor, Batimastat (BB-94). In particular, we show that BB-94 administration to MTB-infected macrophages inhibits apoptosis and the downmodulation of membrane CD14 expression. Moreover, the addition of broad spectrum matrix metalloproteinase inhibitor to cell culture, during MTB infection, decreases the release of soluble TNF-alpha and leads to a simultaneous increase of membrane TNF-alpha. These results show that MTB-induced apoptosis in macrophages is reduced by a MMP inhibitor and most probably is related to TNF-alpha release. This identifies BB-94 as a simultaneous anti-apoptotic and anti-inflammatory molecule during MTB infection.—Authors’ Abstract


Interleukin-15 (IL-15) transgenic mice which had been inoculated with Mycobacterium bovis bacillus Calmette-Guerin (BCG) 24 weeks previously showed resistance against airborne infection with Mycobacterium tuberculosis H37Rv accompanied by an increased CD8(+) Tc1-cell response. IL-15 may be used as an immune adjuvant given with BCG vaccination to enhance its biologic efficacy.—Authors’ Abstract


SUMMARY: OBJECTIVE: Prior to the widespread use of Mycobacterium bovis, Bacille Calmette-Guerin (BCG), inactivated whole cell mycobacterial vaccines had been shown effective in the prevention of tuberculosis. The present study was conducted to determine the safety and immunogenicity of an inactivated whole cell mycobacterial vaccine in persons with HIV infection. DESIGN: Randomized, controlled trial. METHODS: A total of 39 HIV-positive patients with prior BCG immunization and CD4 cell counts ≥200 × 106 cells/l were randomized to five doses of inactivated Mycobacterium vaccae (MV) vaccine or control vaccine (CV). Lymphocyte proliferation (LPA) and interferon gamma (IFN-gamma) responses to mycobacterial antigens were assayed at baseline, after three and five doses of vaccine and >1 year later. Parallel studies were conducted in 10 HIV-negative subjects with prior BCG immunization. RESULTS: Among HIV-positive patients, 19 MV recipients had higher LPA and IFN-gamma responses to MV sonicate than 20 CV recipients after three and five doses of vaccine and >1 year later. LPA responses to Mycobacterium tuberculosis whole cell lysate increased over time in both groups consistent with prior BCG immunization and current antiretroviral therapy; after three doses, responses were boosted to higher levels in MV subjects than CV subjects. LPA responses to WCL were also boosted in HIV-negative MV recipients. Immunization was safe and had no adverse effects on HIV viral load or CD4 cell count. CONCLUSIONS: In BCG-primed, HIV-positive and HIV-negative subjects, MV induces durable cellular immune responses to a new mycobacterial antigen and boosts pre-existing responses to WCL. MV is a candidate for clinical trials for the prevention of HIV-associated tuberculosis.—Authors’ Abstract


SETTING: 1,25-dihydroxyvitamin D3 (1,25(OH)(2)D(3)) is a potent modulator of immune responses and may be beneficial in the treatment of tuberculosis. Recent evidence suggest that 1,25(OH)(2)D(3) may affect T-dependent responses in cattle; however, mechanisms by which this vitamin modulates activation of bovine T cells are unclear. OBJECTIVE: Determine the effects of 1,25(OH)(2)D(3) on the expression of CD25, CD44, and CD62L by bovine T cell subsets proliferating in response to antigen stimulation. DESIGN: Antigen-specific recall responses of Mycobacterium bovis bacille Calmette-Guerin (BCG) vaccinated cattle were used as a model system to evaluate effects of 1,25(OH)(2)D(3) on the proliferation and activation of bovine T cell subsets. RESULTS: CD4(+) and gamma delta TCR(+) cells were the predominant T cell subsets responding to soluble crude M. bovis-derived antigens (i.e., purified protein derivative and a BCG whole cell sonicate) by proliferation and activation-induced alterations in phenotype. These subsets exhibited increased CD25 and CD44 mean fluorescence intensity (mfi) and decreased CD62L mfi upon antigen stimulation. Addition of 1,25(OH)(2)D(3) inhibited proliferation of CD4(+) cells and decreased the expression of CD44 on responding (i.e., proliferating) CD4(+) and gamma delta TCR(+) cells. CONCLUSION: These findings suggest that the production of 1,25(OH)(2)D(3) by macrophages within tuberculous lesions would inhibit proliferation and CD44 expression by co-localized CD4(+) and gamma delta TCR(+) cells.— Authors’ Abstract


This is a descriptive study to assess the leprosy control program in the municipality of Buriticupu in Maranhão State, Brazil. The records of 214 patients with different forms of leprosy were studied. Patients were treated at a health center of the Federal University in Maranhão located in the above-mentioned municipality. The study population was comprised of 110 cases with paucibacillary leprosy (PB) and 104 with multibacillary leprosy (MB). The patients were registered between January 1991 and December 1995. Data on the form of the disease, number of contacts registered, examined, and assessed, degree of disability at the beginning and end of treatment, and the register’s status were collected on a form designed specifically for this purpose. Analysis of results was based on operational guidelines developed by the Ministry of Health. There was a slight predominance of the PB form. Observation of patients with physical disabilities at the beginning and end of treatment was low, as were levels of successful treatment and examined contacts. There was a high dropout level. The program showed “low-level performance” for all indicators used in the study. —Tropical Diseases Bulletin


OBJECTIVE: To evaluate the incidence of adverse reactions to 1st and 2nd BCG vaccination in school children. SETTING AND DESIGN: Enhanced surveillance in a Brazilian trail. Suspected reactions were reported to a nurse who visited cases and
completed a standard form. RESULTS: Among 71,341 school children studied, 33 reactions were reported. Of these, 25 fulfilled the criteria, resulting in a rate of one per 2854 vaccinations, with no deaths or BCG adverse effects. Reactions to 2nd doses were more common than to 1st BCG vaccinations, but this difference was not statistically significant. CONCLUSIONS: Adverse reactions to a 2nd dose of BCG may be more frequent than reactions to a 1st dose, but they are still rare events. —Tropical Diseases Bulletin


A descriptive epidemiological study on the detection of new leprosy cases was conducted in São Luís, Maranhão, Brazil, during 1993–98. A database was created for the purpose, covering 2796 reported cases. General detection rates were calculated, as well as specific rates by gender, clinical type, and age group. Linear, exponential, geometric, and log adjustment models were performed to analyse time trends in the disease. An increase in detection was observed, involving mostly female and paucibacillary cases, mainly of tuberculoid leprosy. The increase in detection was most evident in the age group 15–19 years. The percentage of detection under 15 years indicated the need for active case search in this group. —Tropical Diseases Bulletin


The objective of this study is to assess whether the case-finding method is a determinant for diagnostic characteristics and treatment outcome of newly diagnosed leprosy patients in Northern Mozambique. This is a retrospective cohort study of 3202 patients on the differences between entrance characteristics and treatment outcome in self-reporting patients and patients detected during a leprosy elimination campaign (LEC) in 1999 in Northern Mozambique. As a consequence of LEC activities, 3 times more patients were found compared with the same period 1 year earlier. After the LEC, case detection remained higher in the years 2000–2002 compared with the years preceding the LEC. More young (<15 years) paucibacillary (PB) cases were diagnosed during LEC activities with, surprisingly, equal percentage of disability grades. No gender imbalance was found in diagnosed LEC patients contrary to self-reporting patient groups. Comparing patients detected during a LEC in 1999 with the passive group of 1998 and 1999 showed a slight but statistically significant better treatment result for the passive group. The classification of leprosy (in favor of PB) and age (in favor of older age groups) were also determinants for favorable treatment outcomes. Volunteers had a significantly better result of treatment compared with trained nurses and regardless of detection method. LEC proved to be a useful addition to the National Leprosy and Tuberculosis Programme in Northern Mozambique. As a result, many new cases were diagnosed and put on treatment and their treatment results were very satisfactory. LEC had a lasting impact on case finding. Volunteers make a valuable contribution to leprosy control in Mozambique because they have consistently better treatment results compared with nurses.—Authors’ Abstract


OBJECTIVE: To review the incidence and management of peripheral neuropathy in patients receiving therapy for multiple drug resistant tuberculosis (MDR-TB). METHODS: A case series with retrospective chart review of 75 patients who initiated individualized therapy for MDR-TB in Lima, Peru, between 1 August 1996 and 31 January 1999. RESULTS: All patients had
confirmed MDR-TB and were receiving individualized therapy, comprised of an average of 6 drugs. 10 (13%) of these patients presented with symptoms of peripheral neuropathy, confirmed by electromyography. All symptoms were reported in the lower extremities, and all were sensory in nature. Median time to presentation from initiation of MDR-TB therapy was 9.1 months. No significant risk factors associated with development of peripheral neuropathy were identified. Management strategies depended on the severity of symptoms and included the treatment of contributing co-morbidities, medications for neuropathic pain, and adjustment of doses of possible offending agents. All patients responded to management: 3 patients were left with mild residual symptoms. Patients whose neuropathy resolved had symptoms for a median of 7 months. CONCLUSIONS: Peripheral neuropathy was encountered in 13% of our cohort of MDR-TB patients. The diagnosis of peripheral neuropathy can be based on clinical presentation alone, and effective management of this side effect is possible without sacrificing MDR-TB treatment efficacy. —Tropical Diseases Bulletin


Almost all leprosy cases reported in industrialized countries occur amongst immigrants from developing countries where leprosy continues to be an important health issue. Screening for leprosy is an important question for governments in countries with immigration and refugee programmes. A decision analysis framework is used to evaluate leprosy screening. The analysis uses a set of criteria and parameters regarding leprosy screening, and available data to estimate the number of cases which would be detected by a leprosy screening programme of immigrants from countries with different leprosy prevalences, compared with a policy of waiting for immigrants who develop symptomatic clinical diseases to present for health care. In a cohort of 100,000 immigrants from high leprosy prevalence regions (3.6/10,000), screening would detect 32 of the 42 cases which would arise in the destination country over the 14 years after migration; from medium prevalence areas (0.7/10,000) 6.3 of the total 8.1 cases would be detected, and from low prevalence regions (0.2/10,000) 1.8 of 2.3 cases. Using Australian data, the migrant mix would produce 74 leprosy cases from 10 years intake; screening would detect 54, and 19 would be diagnosed subsequently after migration. Screening would only produce significant case-yield amongst immigrants from regions or social groups with high leprosy prevalence. Since the number of immigrants to Australia from countries of higher endemnicity is not large routine leprosy screening would have a small impact on case incidence.—Authors’ Abstract

Other Mycobacterial Diseases


Between 1995 and 2002, nine cases of nontuberculous mycobacterium (NTM) were isolated from 462 allogeneic stem cell transplant (SCT) recipients (1.9%), and none from 139 autologous cases. They included three cases each of Mycobacterium fortuitum and M. chelonae, and single cases of M. scrofulaceum, M. gordonnae and M. avium complex. Seven cases were respiratory, including five cases requiring treatment, and two involved infected catheters and vascular conduits. Compared with nine cases of Mycobacterium tuberculosis (MTB) isolated in the same period, NTM isolation occurred later after HSCT and involved more unrelated donors. Important risk factors for NTM infection included significant aGVHD (p = 0.043), leukemia relapse (p = 0.022), MUD and mismatch SCT (p <0.001) and existence of BO (p <0.001).
Coinfection with aspergillus was common. Invasive NTM disease required prolonged antimicrobial treatment in five cases due to *M. fortuitum* and *M. chelonae*. With better MTB prophylaxis, intensive immunosuppression and better awareness, NTM has become an emerging threat in oriental allo geneic HSCT recipients. The cutoff between colonization and infection, and the threshold for starting treatment is unclear. NTM isolation is a marker for severe immunosuppression and poor prognosis. When there is doubt over species identity or extent of infection, broad-spectrum cover may be prudent.—Authors’ Abstract


This multicenter, randomized, open-label phase 3 clinical trial compared the safety and efficacy of 3 clarithromycin-containing combination regimens for the treatment of disseminated *Mycobacterium avium* complex (MAC) disease in persons with acquired immunodeficiency syndrome. A total of 160 eligible patients with bacteremic MAC disease were randomized to receive clarithromycin with either ethambutol (C+E), rifabutin (C+R), or both (C+E+R) for 48 weeks. After 12 weeks of treatment, the proportion of subjects with a complete microbiologic response was not statistically significantly different among treatment arms: the proportion was 40% in the C+E group, 42% in the C+R group, and 51% in the C+E+R group (p = 0.454). The proportion of patients with complete or partial responses who experienced a relapse while receiving C+R (24%) was significantly higher than that of patients receiving C+E+R (6%; p = 0.027) and marginally higher than that of patients receiving C+E (7%; p = 0.057). Subjects in the C+E+R group had improved survival, compared with the C+E group (hazard ratio [HR], 0.44; 95% confidence interval [CI], 0.23–0.83) and the C+R group (HR, 0.49; 95% CI, 0.26–0.92).—Authors’ Abstract


A 76-year-old white male presented with progressive malaise, weight loss and dyspnea at rest. Echocardiography revealed a circular pericardial effusion and global hypokinesia. Pericardiocentesis showed a purulent exudate and microbiologic examination revealed *Mycobacterium bovis* fully sensitive to isoniazid, streptomycin, ethambutol, rifampin, and pyrazinamide. By spoligotyping the isolate could be further differentiated to *M. bovis* ssp. caprae. Antimycobacterial therapy was initiated but 3 weeks later the patient’s circulation and renal function deteriorated and he died with clinical signs of sepsis despite intensive care treatment. Pericarditis is a rare manifestation of tuberculosis and can be fatal even when diagnosed and treated appropriately. In low incidence countries diagnosis is often delayed and even overlooked.—Authors’ Abstract


The authors report the case of a male patient who owned a tropical aquarium and who developed a *M. marinum* skin infection of the wrist. The clinical findings and microbiological features of the case are described, as are the difficulty in providing a prompt diagnosis, and the need for surgical treatment and the use of antibiotics to treat the infection.—Authors’ Abstract


A comparison of *Mycobacterium tuberculosis* complex isolates from seals (pinnipeds) in Australia, Argentina, Uruguay, Great Britain and New Zealand was undertaken to determine their relationships to each other and their taxonomic position within the complex. Isolates from 30 cases of tuberculosis in six species of pinniped and seven related isolates were compared to representative and standard strains of the *M. tuberculosis* complex. The seal isolates could be distinguished from other members of the *M. tuberculosis* complex, including the recently defined ‘*Mycobacterium canettii*’ and ‘*Mycobacterium caprae*’, on the basis of host preference and phenotypic and genetic tests. Pinnipeds appear to be the natural host for this ‘seal bacillus,’ although the organism is also pathogenic in guinea pigs, rabbits, humans, Brazilian tapir (Tapirus terrestris) and, possibly, cattle. Infection caused by the seal bacillus is predominantly associated with granulomatous lesions in the peripheral lymph nodes, lungs, pleura, spleen and peritoneum. Cases of disseminated disease have been found. As with other members of the *M. tuberculosis* complex, aerosols are the most likely route of transmission. The name *Mycobacterium pinnipedii* sp. nov. is proposed for this novel member of the *M. tuberculosis* complex (the type strain is 6482(T)=ATCC BAA-688(T)=NCTC 13288(T)).—Authors’ Abstract


A 64-year-old woman with chronic myelogenous leukemia (CML) was admitted due to prolonged fever and lung infiltrates. An open lung biopsy was required to make the diagnosis of pulmonary alveolar proteinosis (PAP) and infection with *Mycobacterium kansasii*. She was treated successfully with combined antimycobacterial therapy for 14 months. However, the leukemia progressed and the patient developed recurrent bilateral lung infiltrates. Blood and bronchoalveolar fluid cultures yielded growth of Acinetobacter. She died shortly thereafter due to septic shock. The relationship between *M. kansasii* infection, PAP, and abnormal host defense in CML is discussed. Copyright 2003 Wiley-Liss, Inc.—Authors’ Abstract


We initiated a prospective trial of an intermittent clarithromycin-containing regimen for the treatment of patients with *Mycobacterium kansasii* lung disease. Eighteen patients (10 men and 8 women) with *M. kansasii* lung disease received a regimen consisting of 500–1000 mg of clar-
ithromycin, 25 mg/kg ethambutol, and 600 mg of rifampin 3 times per week. The primary treatment end point was a 12-month period during which sputum cultures were sterile while the patient was receiving therapy. Four male patients were lost to follow-up, but all of the remaining patients successfully completed therapy without significant drug-related adverse events. The mean time (±standard deviation [S.D.]) to sputum conversion was 1.0 ± 0.9 months, and the mean duration (±S.D.) of therapy was 13.4 ± 0.9 months. No patient who successfully completed therapy had relapsed after a mean (±S.D.) of 46 ± 8.0 months. Clarithromycin- and rifampin-containing regimens offer the possibility of effective short-course and intermittent treatment of *M. kansasii* lung disease.—Authors’ Abstract


The presented case is a 36-year-old woman with a history of systemic lupus erythematosus for 10 years. She had progressively painful swelling of the right index finger that later proved to be a rare case of tenosynovitis caused by *Mycobacterium avium* complex. Serial images of 3-phase bone scans, gallium scan, and magnetic resonance imaging demonstrate the area of involvement.—Authors’ Abstract


To screen effective useful drugs for disease due to *M. abscessus*, we determined MIC of 3 cephems [ceftazidime (CAZ), cefoxitin (CFX), flomoxef (FMOX)] and 3 carbapenems [imipenem (IPM), panipenem (PAPM), meropenem (MEPM)] for 8 strains of clinically isolated *M. abscessus* by micro-dilution method using MGIT system. In all the 8 strains, MICs of CAZ are higher than 32 micrograms/ml. MIC90, MIC range of CFX are 32 micrograms/ml, >32 micrograms/ml and 16 ≥32 micrograms/ml respectively, and for FMOX, 16 micrograms/ml, 32 micrograms/ml and 16–32 micrograms/ml; for IPM, 8 micrograms/ml, 16 micrograms/ml and 8–16 micrograms/ml; for PAPM, 4 micrograms/ml, 16 micrograms/ml and 4–16 micrograms/ml; for MEPM, 8 micrograms/ml, 16 micrograms/ml and 8–16 micrograms/ml. From this study, it is concluded that FMOX, IPM, PAPM and MEPM can be clinically useful drugs in the treatment of the disease due to *M. abscessus*.—Authors’ Abstract


We report treatment decisions and outcomes for 20 patients who were infected with human immunodeficiency virus type 1 (HIV-1) and were receiving highly active antiretroviral therapy (HAART) who had respiratory symptoms and from whom *Mycobacterium xenopi* was isolated. All patients also had coexisting pulmonary pathologic conditions. The median blood T cell CD4 count was 37 cells/microL (range, 2–480 cells/microL). Fifteen of 20 patients received no antimycobacterial therapy and remain healthy after a median of approximately 4 years of follow-up, and 2 patients required treatment specifically for *M. xenopi* infection, both showing clinical improvement. We conclude that pulmonary *M. xenopi* isolation in HIV-1 patients receiving HAART does not usually require specific treatment.—Authors’ Abstract


BACKGROUND: Keratitis due to *Mycobacterium chelonae* after laser-in-situ ker-
atomileusis (LASIK) is a rare, but severe complication. In the following report, we present clinical findings, microbiological investigation, treatment and outcome of the first case of Mycobacterium chelonae reported in Europe. PATIENT AND METHODS: A 52-year-old woman presented with atypical unilateral keratitis after LASIK. Mycobacterium chelonae keratitis was diagnosed by microbiological investigation. Interface irrigation and treatment with topical and oral antibiotics was performed. RESULTS: Despite intensive treatment, flap removal was necessary to control the infection. Best-corrected visual acuity dropped from preoperatively 1.0 to postoperatively 0.2. CONCLUSION: The diagnosis of mycobacterial keratitis after laser-in-situ keratomileusis is often delayed due to atypical clinical appearance. Therefore consideration of atypical pathogens and rapid microbiological diagnosis is necessary to provide adequate treatment. —Authors’ Abstract


We report a woman in whom a slow-growing scotochromogenic strain of Mycobacterium was cultured from skin lesions. According to its phenotypic and biochemical characteristics we could predict only that it might be M. szulgai, M. scrofulaceum or M. gordonae. Polymerase chain reaction amplification of the hsp65 gene and subsequent restriction fragment length polymorphism analysis on the isolated strain showed that its restriction pattern differed from both M. scrofulaceum and other scotochromogenic species. Ninety-nine per cent similarity was detected between the isolated strain and M. gordonae by sequencing of the hsp65 gene. This result suggests that the isolated strain may be either a slow-growing scotochromogenic Mycobacterium most resembling M. gordonae or a novel mycobacterial species. —Authors’ Abstract


BACKGROUND AND SETTING: A reliable and timely clinical, radiological, and bacteriological diagnosis, and an optimal treatment of non-tubercular mycobacteriosis (including Mycobacterium xenopi disease), remain an unanswered challenge for clinicians facing immunocompromised patients, including those with HIV infection. OBJECTIVE: The aim of our survey is to report the frequency, and the epidemiological, immunological, microbiological, clinical, and therapeutic features of all confirmed HIV-associated M. xenopi disease observed from 1993–2002, with special attention paid to eventual differences that emerged after the introduction of potent antiretroviral therapy (highly active antiretroviral therapy, HAART), on the basis of an international literature update. DESIGN AND RESULTS: Our series of 17 consecutive confirmed M. xenopi infections retrieved in 14 out of 3000 patients followed for HIV disease complications raises a broad series of clinical, diagnostic, therapeutic, and prophylactic concerns. The great majority of M. xenopi disease involved the lower respiratory tract, but atypical features including cavitation and prominent exudative features became apparent in patients successfully treated with HAART, pointing out the possible role of the so-called immune reconstitution syndrome in these episodes. CONCLUSIONS: Diagnostic problems represented by late or missed identification due to slow culture and frequently concomitant opportunistic disorders, join therapeutic difficulties due to the unpredictable in vitro antimicrobial susceptibility profile of these organisms, selection of treatment and chemoprophylaxis according with clinical-radiological and microbiological suspicion, and concomitantly administered medications. —Authors’ Abstract

Mycobacterium ulcerans is an environmental pathogen concerning mainly the tropical countries; it is the causative agent of Buruli ulcer, which has become the third most important mycobacterial disease. In spite of water-linked epidemiological studies to identify the sources of \textit{M}. \textit{ulcerans}, the reservoir and the mode of transmission of this organism remain elusive. To determine the ecology and the mode of transmission of \textit{M}. \textit{ulcerans} we have set up an experimental model. This experimental model demonstrated that water bugs were able to transmit \textit{M}. \textit{ulcerans} by bites. In insects, the bacilli were localized exclusively within salivary glands, where it could both multiply contrary to other mycobacteria species. In another experimental study, we report that the crude extracts from aquatic plants stimulate in vitro the growth of \textit{M}. \textit{ulcerans} as much as the biofilm formation by \textit{M}. \textit{ulcerans} has been observed on aquatic plants. Given that the water bugs are essentially carnivorous, it is difficult to imagine a direct contact in the contamination of aquatic bugs and plants. It seems very likely that an intermediate host exists. In an endemic area of Daloa in Cote d’Ivoire, our observations were confirmed.—Authors’ Abstract


\textit{Mycobacterium avium} is an important pathogen among immunodeficient patients, especially patients with AIDS. The natural history of this disease is unclear. Several environmental sources have been implicated as the origin of this infection. Polyclonal infection with this species is observed, challenging the understanding of its pathogenesis and treatment. In the present study 45 \textit{M}. \textit{avium} strains were recovered from 39 patients admitted to a reference hospital between 1996 and 1998. Species identification was performed using a species-specific nucleic acid hybridization test (AccuProbe) from Gen-Probe. Strains were genotyped using IS1245 restriction fragment length polymorphism typing. Blood was the main source of the organism. In one patient with disseminated disease, \textit{M}. \textit{avium} could be recovered more than once from potentially sterile sites. Strains isolated from this patient had different genotypes, indicating that the infection was polyclonal. Four patient clones were characterized in this population, the largest clone being detected in eight patients. This finding points to a common-source transmission of the organism.—Authors’ Abstract


A 61-year-old previously healthy man developed chronic dermal granulomata in his right arm after receiving a coral injury in Thailand. After 7 biopsies, infection caused by \textit{Mycobacterium haemophilum} was diagnosed. This case highlights the difficulty of isolating this fastidious organism in the laboratory and suggests that seawater or coral was the source of the infection.—Authors’ Abstract


OBJECTIVES: A series of cases infected with rapidly growing mycobacteria was studied to determine the spectrum of disease, antimicrobial susceptibility, treatment, and outcome. METHODS: The cases identified as infections with rapidly growing mycobacteria in Ramathibodi Hospital from January 1993 to December 1999 were retrospectively studied. RESULTS: Most of the cases had no underlying disease. Only two cases were HIV-infected patients. The presenting clinical features were lym-
phadenitis (seven cases), skin and/or subcutaneous abscess (seven cases), localized eye infection (four cases), pulmonary infection (one case), and chronic otitis media (one case). Four of seven cases with lympha denitis had Sweet’s syndrome, and one had psoriasis as an associated skin manifestation. Anemia was present in five cases, and improved with treatment of the primary disease. The organisms were *Mycobacterium chelonae* /abscessus group (17 cases) and *Mycobacterium fortuitum* group (three cases). Susceptibility patterns of the organisms showed susceptibility to amikacin, netilmicin, and imipenem. *M. fortuitum* group was susceptible to more antibiotics than *M. chelonae*/abscessus group. The clinical responses corresponded to the antimicrobial susceptibility. Combinations of two or more drugs were used for the medical treatment. Surgical resection was performed where possible, to reduce the load of the organism, especially in cases with very resistant organisms. CONCLUSIONS: Infections with rapidly growing mycobacteria can occur in apparently normal hosts. The clinical syndrome is variable. The pathology is nonspecific. Clinical responses varied, but seemed to correlate with the in vitro susceptibility result. More studies are needed to enable us to deal with this infection effectively.—Authors’ Abstract


OBJECTIVES: A series of cases infected with rapidly growing mycobacteria was studied to determine the spectrum of disease, antimicrobial susceptibility, treatment, and outcome. METHODS: The cases identified as infections with rapidly growing mycobacteria in Ramathibodi Hospital from January 1993 to December 1999 were retrospectively studied. RESULTS: Most of the cases had no underlying disease. Only two cases were HIV-infected patients. The presenting clinical features were lymphadenitis (seven cases), skin and/or subcutaneous abscess (seven cases), localized eye infection (four cases), pulmonary infection (one case), and chronic otitis media (one case). Four of seven cases with lymphadenitis had Sweet’s syndrome, and one had psoriasis as an associated skin manifestation. Anemia was present in five cases, and improved with treatment of the primary disease. The organisms were *Mycobacterium chelonae* /abscessus group (17 cases) and *Mycobacterium fortuitum* group (three cases). Susceptibility patterns of the organisms showed susceptibility to amikacin, netilmicin, and imipenem. *M. fortuitum* group was susceptible to more antibiotics than *M. chelonae* /abscessus group. The clinical responses corresponded to the antimicrobial susceptibility. Combinations of two or more drugs were used for the medical treatment. Surgical resection was performed where possible, to reduce the load of the organism, especially in cases with very resistant organisms. CONCLUSIONS: Infections with rapidly growing mycobacteria can occur in apparently normal hosts. The clinical syndrome is variable. The pathology is nonspecific. Clinical responses varied, but seemed to correlate with the in vitro susceptibility result. More studies are needed to enable us to deal with this infection effectively.—Authors’ Abstract


Reported here are three cases of pulmonary *Mycobacterium xenopi* infection that occurred in AIDS patients in Hungary shortly after starting highly active antiretroviral therapy. In this country, *Mycobacterium xenopi* is the most common nontuberculous mycobacterial species causing pulmonary mycobacterial infections. Cases of pulmonary *Mycobacterium xenopi* disease have been described in patients infected with the human immunodeficiency virus infection and in patients with other immunodeficiencies; however, only limited information is currently available concerning the connection between nontuberculous *Mycobacterium infection* and AIDS in Hungary. This report thus adds useful information regarding the diagnosis, clinical course, and
treatment regimens of *Mycobacterium xenopi* infections in AIDS patients.—Authors’ Abstract


**CONTEXT:** *Mycobacterium ulcerans* causes devastating necrotic lesions in affected individuals. The disease, commonly called Buruli ulcer, is increasing in prevalence in western African countries. Treatment is mainly surgical; no clinical trials have been done to support the use of antimycobacterial drugs. A secreted polyketide toxin, mycolactone, is responsible for the tissue damage; its chemical structure has been elucidated. **STARTING POINT:** Although the main treatment is surgical, many patients with Buruli ulcer present late because of unusual beliefs about the disease and its treatment. Isabelle Aujoulat and colleagues recently showed, in a study in southern Benin, Africa (Trop Med Int Health 2003; 8: 750–759), that although the ulcer is well recognized, the cause is often seen as environmental or because of witchcraft. In addition, treatment is thought to be destructive, costly, and ineffective. **WHERE NEXT?** Antimycobacterial drug regimens that hold promise based on animal and preliminary human studies will soon be tested in large well-designed controlled clinical trials. Information gleaned from the genomic sequence of *M. ulcerans* could be used to design more effective vaccines, or new drug targets (e.g., that knock out the enzymes of *M. ulcerans* that synthesize mycolactone species).—Authors’ Abstract

**Molecular and Genetic Studies**


Leprosy presents as a clinical and immunological spectrum of disease. With the use of gene expression profiling, we observed that a distinction in gene expression correlates with and accurately classifies the clinical form of the disease. Genes belonging to the leukocyte immunoglobulin-like receptor (LIR) family were significantly up-regulated in lesions of lepromatous patients suffering from the disseminated form of the infection. In functional studies, LIR-7 suppressed innate host defense mechanisms by shifting monocyte production from interleukin-12 toward interleukin-10 and by blocking antimicrobial activity triggered by Toll-like receptors. Gene expression profiles may be useful in defining clinical forms of disease and providing insights into the regulation of immune responses to pathogens.—Authors’ Abstract


We developed schemes for rapid identification of *Mycobacterium* species and strain typing using a microfluidic labchip instrument. A 439-bp region of the gene that codes for the 65-kDa heat shock protein (hsp65), which has sequence polymorphisms specific for most mycobacterial species, was examined using PCR-restriction analysis (PRA). We performed PRA in duplicate, using 2 strains each of 12 species, and observed that fragment sizes (bp) determined automatically by the instrument were consistently smaller than the correct sizes for each of the species as determined by sequence analysis (mean variance, <7 bp). *Mycobacterium tuberculosis* isolates were
typed with the labchip instrument using mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing, which determines the number of copies of repeated units at 12 loci in the genome based on product size after PCR amplification. Seven strains with one to six repeat copies at each locus were examined. Sizes were smaller by a mean of 13.47 bp compared with correct sizes predicted by sequence analysis, but could be used to correctly identify all strains types. Isolates of Mycobacterium chelonae and Mycobacterium abscessus were typed using randomly amplified polymorphic DNA (RAPD) electrophoresis, and patterns obtained using the labchip instrument were compared with multilocus enzyme electrophoresis (MEE) types. Patterns were distinct and reproducible for all strains except those with closely related MEE types. The labchip instrument is a versatile alternative for sizing mycobacterial DNA fragments.—Authors’ Abstract


A 9.5-kb section of DNA called region of deletion 1 (RD1) is present in virulent Mycobacterium tuberculosis strains but is deleted in all attenuated Mycobacterium bovis BCG vaccine strains. This region codes for at least nine genes. Some or all RD1 gene products may be involved in virulence and pathogenesis, and at least two, ESAT-6 and CFP-10, represent potent T- and B-cell antigens. In order to produce the entire set of RD1 proteins with their natural post-translational modifications, a robust expression system for Mycobacterium tuberculosis proteins in the fast-growing saprophytic strain Mycobacterium smegmatis was developed. Our system employs the inducible acetamidase promoter and allows translational fusion of recombinant Mycobacterium tuberculosis proteins with polyhistidine or influenza hemagglutinin epitope tags for affinity purification. Using eGFP as reporter gene, we showed that the acetamidase promoter is tightly regulated in M. smegmatis and that this promoter is much stronger than the widely used constitutive groEL2 promoter. We then cloned 11 open reading frames (ORFs) found within RD1 and successfully expressed and purified the respective proteins. Sera from tuberculosis patients and M. tuberculosis-infected mice reacted with 10 purified RD1 proteins, thus demonstrating that Rv3871, Rv3872, Rv3873, CFP-10, ESAT-6, Rv3876, Rv3878, Rv3879c and ORF-14 are expressed in vivo. Finally, glycosylation of the RD1 proteins was analyzed. We present preliminary evidence that the PPE protein Rv3873 is glycosylated at its C terminus, thus highlighting the ability of M. smegmatis to produce M. tuberculosis proteins bearing posttranslational modifications.—Authors’ Abstract


The growing list of fully sequenced genomes, combined with innovations in the fields of structural biology and bioinformatics, provides a synergy for the discovery of new drug targets. With this background, the TB Structural Genomics Consortium has been formed. This international consortium is comprised of laboratories from 31 universities and institutes in 13 countries. The goal of the consortium is to determine the structures of over 400 potential drug targets from the genome of Mycobacterium tuberculosis and analyze their structures in the context of functional information. We summarize the efforts of the UCLA consortium members. Potential drug targets were selected using a variety of bioinformatics methods and screened for certain physical and species-specific properties to yield a starting group of protein targets for structure determination. Target determination methods include protein phylogenetic profiles and Rosetta Stone methods, and the
use of related biochemical pathways to select genes linked to essential prokaryotic genes. Criteria imposed on target selection included potential protein solubility, protein or domain size, and targets that lack homologs in eukaryotic organisms. In addition, some protein targets were chosen that are specific to *M. tuberculosis*, such as PE and PPE domains. Thus far, the UCLA group has cloned 263 targets, expressed 171 proteins and purified 40 proteins, which are currently in crystallization trials. Our efforts have yielded 13 crystals and eight structures. Seven structures are summarized here. Four of the structures are secreted proteins: antigen 85B; MPT 63, which is one of the three major secreted proteins of *M. tuberculosis*; a thioredoxin derivative Rv2878c; and potentially secreted glutamate synthetase. We also report the structures of three proteins that are potentially essential to the survival of *M. tuberculosis*: a protein involved in the folate biosynthetic pathway (Rv3607c); a protein involved in the biosynthesis of vitamin B5 (Rv3602c); and a pyrophosphatase, Rv2697c. Our approach to the *M. tuberculosis* structural genomics project will yield information for drug design and vaccine production against tuberculosis. In addition, this study will provide further insights into the mechanisms of mycobacterial pathogenesis.—Authors’ Abstract


Based on their immunodominant nature and ability to induce appropriate immune responses in the host, several antigens of *Mycobacterium tuberculosis* have shown promise of protection. However, most of the candidate vaccines developed by employing various strategies have afforded protection that is at best comparable with bacillus Calmette-Guerin (BCG) in animal models. Due to the inherent ability of BCG to prime cellular responses in the host, it has become an attractive vehicle for development of a vaccine against intracellular infections. In this study, we have cloned the genes of three immunodominant antigens of *M. tuberculosis* viz. the ESAT6 (Rv3875), the 19 kDa lipoprotein (Rv3763) and the 38 kDa antigen (Pst homolog) (Rv0934) in pSD5 under the transcriptional control of Trm, a strong mycobacterial promoter, and expressed them in BCG. The 19 kDa antigen and the 38 kDa antigen were expressed at levels that were approximately five and eightfolds higher in the cytosols of recombinant BCG strains rBCG19T and rBCG38T, respectively, as compared with their corresponding levels in *M. bovis* BCG. Both these antigens were also secreted into the extracellular medium at enhanced levels (19 kDa antigen fourfold and 38 kDa antigen twofold) by rBCG strains in comparison with the wild type BCG. ESAT6 antigen, which is absent in *M. bovis* BCG, was also expressed at a very high level in the cytosol of the rBCG strain (rBCGE6T). Evaluation of immune responses induced by these three rBCG strains in mice shows a markedly different pattern. The rBCG strain overexpressing the 38 kDa antigen exhibited a predominant T helper 1 (Th1) response with high levels of interferon-gamma (IFN-gamma) production, whereas overexpression of the 19 kDa antigen resulted in completely polarized Th2 responses against the BCG sonicate. The rBCG-expressing ESAT6 antigen induced a mixed Th1/Th2 response. Our observations suggest that the 38 kDa antigen may hold excellent promise in the rBCG approach for the development of a vaccine against tuberculosis.—Authors’ Abstract


The type strain of *Mycobacterium simiae* and four Cuban strains, each representing a group of variants sharing a characteristic pattern of glycopeptidolipids, were investigated. Each of the five strains was found to have a single rRNA (rrn) operon per genome. Each rrn operon was found to be
located downstream from murA. Unusually for slow-growing mycobacteria, three transcription start points were identified for each operon. Gene sequences were established extending from near to the 3′-ends of murA, the intergenic regions and the 5′-ends of the 16S rDNAs. Characteristic strain differences were identified.—Authors’ Abstract


Despite the importance of tuberculosis as a public health problem, we know relatively little about the molecular mechanisms used by the causative organism, *Mycobacterium tuberculosis*, to persist in the host. To define these mechanisms, we have mutated virtually every nonessential gene of *M. tuberculosis* and determined the effect disrupting each gene on the growth rate of this pathogen during infection. A total of 194 genes that are specifically required for mycobacterial growth in vivo were identified. The behavior of these mutants provides a detailed view of the changing environment that the bacterium encounters as infection proceeds. A surprisingly large fraction of these genes are unique to mycobacteria and closely related species, indicating that many of the strategies used by this unusual group of organisms are fundamentally different from other pathogens.—Authors’ Abstract


See Current Literature, Immuno-pathology, Leprosy, p. 82.


Fold recognition was applied to the systematic analysis of the all sequences encoded by the genome of *Mycoplasma tuberculosis* H37Rv in order to identify new putative glycosyltransferases. The search was conducted against a library composed of all known crystal structures of glycosyltransferases and some related proteins. A clear relationship appeared between some sequences and some folds. It appears necessary to complete the fold recognition approach with a statistical approach in order to identify the relevant data above the background noise. Exploratory data analysis was carried out using several methods. Analytical methods confirmed the validity of the approach, while predictive methods, although very preliminary in the present case, allowed for identifying a number of sequences of interest that should be further investigated. This new approach of combining bioinformatics and chemometrics appears to be a powerful tool for analysis of newly sequenced genomes. Its application to glycomics is of great interest.—Authors’ Abstract


The arabinans of the mycobacterial cell wall are key structural and immunological polymers in the context of arabinogalactan (AG) and lipoarabinomannan (LAM) respectively. The three homologous membrane proteins EmbA, EmbB and EmbC are known to be involved in the synthesis of arabinan but their biochemical functions are not understood. Herein we show, that synthesis of LAM, but not AG, ceases after inactivation of embC in *Mycobacterium smegmatis* by insertion mutagenesis. LAM synthesis is restored upon complementation with the embC wild-type gene.
Previously we have shown that the synthesis of the arabinan of AG is affected by embA or embB disruption. Thus the Emb proteins are capable of differential recognition of the galactan or mannan acceptors prior to appropriate arabinosylation. In addition, a combination of genetic and biochemical approaches have allowed us to assign some specific functions to the regions of emb gene products. Complementation of the embCmacr; mutant with a hybrid gene encoding the N-terminus of EmbC and the C-terminus of EmbB resulted in LAM with a lower molecular weight than the wild-type LAM. Structural studies involving enzyme digestion, chromatography and mass spectrometry analyses revealed that the arabinan of the ‘LAM’ formed in the hybrid was of AG kind rather than LAM type of arabinan.—Authors’ Abstract
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