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Ulcerative Cutaneous Mycobacteriosis Due to *Mycobacterium ulcerans*: Report of Two Mexican Cases

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ABSTRACT

We report two patients from Central Mexico, with ulcerated cutaneous lesions containing acid-fast bacilli (AFB) and ultimately diagnosed as *Mycobacterium ulcerans* disease. The first patient had a long history (11 years) of disease involving multiple lesions of both upper and lower extremities. Histopathological changes included necrosis of the subcutaneous tissue with large numbers of extracellular AFB. Cultures at 32°C were "positive for mycobacteria," but were not further identified. The polymerase chain reaction for *M. ulcerans* performed on skin biopsies was positive. The lesions improved after treatment with rifampin and isoniazid (INH) for one month, followed by ethambutol and streptomycin.

The second case followed trauma to the right hand, which spread over 2 years to the right upper extremity, the back, and both legs, with a loss of digits and metacarpal bones of the right hand. The histopathological findings were similar to the first case, including presence of AFB. PCR for *M. ulcerans* on extracts of skin biopsies was positive. Rifampin, INH, pyrazinamide, and levofloxacin resulted in marked improvement of the ulcer; ethambutol and streptomycin were later used, also.

We report these cases because they are rare (approximately 6 previous cases were reported from Mexico), and both are unusually disseminated. They are significant in alerting the medical community to *M. ulcerans* infection, which is still active in Mexico, and the treatment used has not been reported previously.

RÉSUMÉ

Cet article décrit la maladie de deux patients habitant la région centrale du Mexique, qui souffraient de lésions cutanées ulcérées contenant des bacilles acido-alcoolo-résistants (AAR) et qui ont finalement été diagnostiqués comme souffrant de maladie causée par

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Case 1. A 76-year-old woman, occasional farmer, born and lived in Esperanza Tarimoro, (Guanajuato, Central Mexico), presented in 1995 with an eleven-year history of an evolving dermatosis, affecting the left forearm and elbow, index and middle fingers and back of the right hand, the left thigh on its anterior and lower sides, the knee, and posterior mid-left leg. The lesions consisted of 10 nodules between 2 to 4 cm in diameter, red-violet in color, with a hard consistency, and non-pitting edema. Some also had ulcerations and were draining bloodstained serum at the center (gummae). Seven ulcers measured 2 to 10 cm in diameter had open, undermined borders that allowed the introduction of a clamp. The underside was necrotic and partially covered with purulent secretion (Fig. 1, Case 1). At the right knee were three circular scars, 1
cm in diameter, with erythema and marked swelling.

At the onset there were small, erythematous nodules on the legs. Some of these had healed spontaneously one year earlier, and new lesions had appeared on her arms, which ulcerated after 9 mos. Previous treatments included home remedies. The initial clinical diagnosis was deep nodular tuberculosis (Hutchinson type). General medical examination revealed a patient in good general condition. Laboratory analysis showed aleukocytosis with 14,900 white blood cells (83% segmented cells), an incremented globular sedimentation rate of 25 mm/hr, and normal hepatic function and chest x-rays. The skin test with 5TU purified protein derivate (PPD) was negative.

Two skin biopsies were taken, one from the border of the ulcer on the left forearm and the other from the nodule on the left thigh. Histologically, we observed large necrosis zones affecting the middle and deep dermis and hypodermis in both biopsies (Figs. 2 and 3). Fite-Faraco stains revealed large numbers of acid-fast bacilli (AFB) in necrotic areas, some in clusters and forming “globi,” (Figs. 4 to 7), similar to those described by MacCallum (15) and Connor(28).

Further analysis of an acid-fast smear of the purulent secretion from borders and bottom ulcers, using a Ziehl Neelsen stain, revealed clusters of AFB. Cultures on Lowenstein-Jensen media at 32°C were positive for mycobacteria. The cultures were damaged before further identification could be obtained from a referral center. The final diagnosis was Ulcerative Cutaneous Mycobacteriosis (UCM), species unknown.

Treatment with rifampin 600 mg/day and isoniazid 300 mg/day, and daily soaks of ul-
cers in sulfate solution (1:1000) for one month resulted in improvement. Rifampin was discontinued after the patient developed clinical hepatitis attributable to this drug. Two weeks later, after hepatic function tests returned to normal, treatment was initiated with ethambutol 600 mg/day and streptomycin 1 gm/day for 15 days, then reduced to 1 gm every three days, for a total of 30 gr. With this regimen, the ulcers healed. At this stage the patient returned to her hometown and was lost to follow-up.

**Case 2.** A 23-year-old male horse-meat merchant who lived in Chimalhuacán, Mexico (Central Mexico), presented with a 2-year evolving dermatosis, affecting the lower right arm and elbow, forearm, and dorsum of the right hand, anterior aspect of both legs, and posterior aspect of the left leg. There were four nodules of 2 cm in diameter and 10 ulcers between 1 and 5 cm in diameter with the characteristics described for the first case. Some ulcers were communicating and alternated with small areas of apparently normal skin. The largest ulcerative lesion was on the forearm, measuring 15 × 25 cm in diameter, with viscous crust, surrounded by scar tissue (Fig. 8), in the area previously grafted. Scars were present, 3 cm in diameter. We also observed absence of all of the fingers of the right hand, with exception of the thumb and the metacarpal bones.

The disease presented after trauma to the right hand from a prick with a horse bone chip; 15 days later, there was reddening and swelling of the area that quickly extended to the forearm. At the trauma site, the patient observed necrosis, which was excised, but despite this the disease continued. The patient was then sent to the Instituto Nacional de Ortopedia in Mexico City in August 1997, where the forearm lesion was excised, four fingers were amputated, and the metacarpal zone was covered with a skin graft. Results were poor and the disease continued. Two years later, the patient was sent to the Centro Dermatologico Pas- cua (CDP), where the initial clinical diagnosis was cutaneous tuberculosis. The skin test with STU PPD showed 5 mm of induration, and the physical examination was otherwise normal. With the history of trauma precedents, long evolution, and poor response to the prescribed treatment, we considered the possibility of a diagnosis of UCM.

Four biopsy specimens were taken from the necrotic area on the ulcers of the fore-
arm and leg nodules. Microscopic findings were similar in all specimens and identical to those previously described in Case 1, confirming the clinical diagnosis.

Bacilloscopies revealed AFB and an enzyme-linked immunosorbant assay (ELISA) was positive for *Mycobacterium* sp., but cultures for mycobacteria were negative. Routine laboratory determinations were within the normal range.

Surgical cleaning and soaks with sulfate solutions (1:1000) were carried out twice a day. Rifampin 600 mg/day, isoniazid 300 mg/day, pyrazinamide 300 mg/day and levofloxacin 400 mg/day, were administrated for two months, with great improvement of the ulcers, marked by a decrease of purulent drainage and necrosis. The nodules, however, persisted. Surgical excision of nodules was performed and microscopic analysis showed necrotic zones and numerous acid-fast bacilli. Streptomycin 1 gr intramuscularly (IM) every three days (30 gr total dose) and ethambutol 1200 mg/day were then administered. Additionally, surgical cleaning was performed once a week and topical soaks with sulfate solutions was performed daily. After streptomycin was discontinued, rifampin 600 mg/day was resumed with ethambutol 1200 mg/day again for 10 months, and the cutaneous lesions healed. (Fig. 9). At present, the patient is under periodic monitoring and shows no lesions, and a prosthesis has been fitted to the patient’s right hand.

Paraffin blocks of biopsies from both patients were sent to Dr. Francois Portaels at the Institute for Tropical Medicine, Antwerp, Belgium, for analysis by polymerase chain reaction. Specimens from both patients were found to contain DNA sequence IS2404 from *M. ulcerans*.

**DISCUSSION**

The skin ulceration caused by *Mycobacterium ulcerans* was described for the first time by MacCallum, *et al.* in Australia in 1948 (16). In 1950 in the Belgian Congo (now the Democratic Republic of Congo) the first African case was reported (29), and in the same year, Fenner (7) identified the bacillus and named it *Mycobacterium ulcerans*. Since 1959, several authors have described numerous patients with this disease in tropical and subtropical regions of Central and West Africa (13). Buruli ulcer is recognized as a public health problem, for example, in Uganda, Nigeria, Gabon, Ghana, Cameroon, Liberia, the Ivory Coast (1, 6, 17, 28, 30), Malaysia (22), New Guinea (14), Togo (15), French Guyana (5, 25), and the Republic of Benin (20). In the Americas, it is an exceptionally rare disease and only a few cases have been reported. In 1953, Lavalle, *et al.*, reported the first UCM case in Mexico (11, 13) and until 1990, only five additional cases from Guanajuato State in Central Mexico were reported (13, 14, 15).

This mycobacteriosis has been given several names according to the place where it occurs or where it has been observed. For example, it was called Bairnsdale ulcer in Australia (17), Buruli ulcer in Uganda (20), and Tora ulcer and Mexican ulcer in México (19). Nevertheless, Lavalle proposed the name of Ulcerative Cutaneous Mycobacteriosis, caused by *Mycobacterium ulcerans* (15).

*Mycobacterium ulcerans* is a slowly grow-
ing, acid-fast organism generally considered to be an environmental saprophyte. It is usually observed in aquatic ecosystems in marshy terrain, a soil rich in silica, and in stagnant bodies of water or near rivers, at temperatures ranging between 32°C and 33°C, with pH between 5.5 and 6.9 (8,20,21). The bacillus grows best in Lowenstein-Jensen culture medium at 32°C.

The disease affects both sexes and all ages, but is more frequent in children between 5 and 14 yrs old (29). In the countries where it is endemic, it is frequent in farmers and may be considered an occupational disease. After tuberculosis and leprosy, M. ulcerans infection is considered the third most common mycobacterial disease affecting non-immunocompromised humans (20).

The exact manner of transmission of M. ulcerans is not known. It is assumed that a not-yet identified environmental factor exists that is related to slowly flowing or stagnant water and near rivers. There are some reports suggesting possible transmission by mosquito or insect bites (3,4,24). However, inoculation appears to occur via trauma to skin, on uncovered, unprotected regions of the body. Its topography in adults includes limbs, especially near joints, predominantly on legs (knees) and forearms (elbows) (5,8,19), but in children it can be found anywhere. M. ulcerans affects humans by producing a heat-stable exotoxin that causes extensive, chronic, necrotizing damage to the papillary skin, subcutaneous fat and muscle (fascia and bone are also sometimes affected), resulting in deformity and disability (12).

The lesion begins as a small subcutaneous swelling, more palpable than visible, that grows slowly until it develops into a nodule that is adherent to the skin but not to deep tissues. These nodules are soft, undergo liquefaction, and finally ulcerate, with an oily, purulent discharge. Ulcers are often well defined and the borders are undermined. The base of the pristine ulcers contains a whitish, cotton wool-like slough and sometimes eschars. Skin surrounding the lesion becomes hyper-pigmented (15). Ulcers can be small or extensive, involving even an entire extremity or large portions of the trunk. Microscopic alterations are usually diagnostic, including extensive necrosis of the dermis and large numbers of extracellular AFB, in clumps or clusters (2,6,16).

Disease evolution can vary in severity. In some areas, ulcers heal slowly with fibrosis and retraction, even while the disease may progress in other areas. Secondary bacterial infection may develop, but the patient’s general condition is not affected. There is no regional clinical lymphadenopathy nor fever.

The two cases described in this report are particularly interesting because of the unusual dissemination of the disease and the large number of nodules and ulcers. The possible mode of transmission was not apparent in the first case, whereas in the second case, the initial lesion followed trauma with a horse bone chip, similar to one of the Lavalle cases (15). This information allows us to suspect that a direct inoculation was made in this case.

The last report of Buruli ulcer the disease in Mexico was made several years ago, and no reports of other cases have been made since. This may be attributable to possible rarity of the disease in Mexico. As Lavalle suggests, the paucity of reports may be a result of a lack of awareness of the disease, or to the status of the public health services in endemic areas. Innate or acquired immunity of the populations may also contribute to low endemicity.

We conclude that the following features must be considered for the diagnosis of M. ulcerans infection: (i) a chronic dermatosis in a patient with good general health; (ii) the histopathological findings of extensive necrosis in the dermis and subcutaneous tissue and the presence of numerous extracellular acid-fast organisms (2,5,4).

Although it is difficult to culture this organism, it is now possible to identify the agent by PCR analysis carried out on the skin specimen (10,20), although in our opinion these studies are not necessary for the diagnosis. In both cases presented in this report, PCR analysis for M. ulcerans DNA was performed on paraffin blocks of the skin biopsies, and these studies were done some years after the diagnoses were made. The treatments were initiated on the basis of clinical and histopathological findings, and the patients healed. This indicates that the molecular studies are not indispensable if there is an adequate clinical and histological study, but PCR contributes to support the diagnosis by identifying the mycobacterial DNA sequence IS2404.
Despite the fact that the first cases were adequately described more than 50 years ago, there remains no standard effective treatment. Surgical excision of skin lesions and, if necessary, skin graft application in the initial stages, are considered the best treatment, in addition to anti-mycobacterial agents. Hyperbaric oxygenation has been used experimentally (26). This schedule of antimicrobial treatment was applied in Case 2 as described in this report, obtaining a complete recovery after 12 months. The regimens used for treatment in both patients have not been reported previously, and they give affected patients the possibility of healing without important sequelae such as amputation. Additional preventive efforts, such as BCG vaccination (23) and wearing long pants in endemic regions to protect molecular biology (PCR) studies.

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A Delphi Consensus on Criteria for Contraindications, Assessment Indicators and Expected Outcomes Related to Tibialis Posterior Transfer Surgery

Hugh Cross

ABSTRACT

A team of experts in the field of reconstructive surgery for leprosy-affected people was identified. Using the Delphi method, an exercise was undertaken to ascertain whether a consensus on essential criteria and indicators for Tibialis Posterior Transfer (TPT) could be reached among the team. This paper describes the Delphi Exercise, giving results at each stage of consensus development. The final outcome was that essential criteria, including contraindications for surgery, pre- and post-operative assessments and expected outcomes, were agreed. The criteria are presented with recommendations.

RÉSUMÉ

Un groupe d’experts en chirurgie reconstructrice a été mis en place pour les patients souffrant de lepré. En utilisant la méthode de Delphi, un exercice a été entrepris afin de vérifier si un consensus pouvait être atteint au sein du groupe au sujet des critères essentiels d’indication pour un Transfert du Tibialis Postérieur (TTP). Cet article décrit cet exercice selon la Méthode de Delphi et présente les résultats à chaque étape du développement du consensus. Le résultat final a été qu’un accord général a été obtenu sur des critères essentiels comme les contre-indications à la chirurgie, les évaluations pré et post-opératoires et les résultats attendus. Les critères sont présentés avec des recommandations.

RESUMEN

Para este estudio se contactó a un equipo de expertos en el campo de la cirugía reconstructiva para personas afectadas de lepra. Usando el método Delphi, se realizó un ejercicio para saber si el equipo podía llegar a un consenso sobre los criterios e indicadores esenciales para la Transferencia Tibial Posterior (TTP). En este artículo se describe el ejercicio de Delphi y se proporcionan los resultados obtenidos en cada etapa del desarrollo del consenso. El resultado final del ejercicio fue que hubo concordancia en los criterios esenciales, incluyendo las contraindicaciones de la cirugía, las valoraciones pre- y post-operatorias, y los resultados esperados. Se presentan los criterios y las recomendaciones del estudio.

It is generally agreed that treatment for leprosy is best integrated into the general health service provision. However, anecdotal reports from the field suggest that the decline in registered leprosy prevalence is impacting the scope of service provisions and the accessibility of referral services.

The declining investment in leprosy may also impact professionals seeking to develop challenging careers. High profile health and development issues (e.g., AIDS, environmental challenges, etc.), which arouse wide public awareness and elicit strong financial support are likely to attract professional interest away from leprosy. The dilemma is compounded because high profile health and development issues, which arouse wide public awareness and elicit strong financial support, attract the interest of professionals seeking to develop challenging careers. Furthermore, much of the invaluable clinical experience and expertise that has hitherto been a resource to field programs is invested in relatively few exceptional people,
many of whom are already embracing retirement. The transfer of knowledge and practice to ensure that a sound core of expertise is maintained is an issue. There is a considerable body of published evidence upon which a variety of clinical developments can be based, but certain procedures do not readily lend themselves to empirical investigation (e.g., criteria for assessments or the grading of outcomes). There is a compulsion to act expeditiously so that the wealth of wisdom and experience that does still exist may be tapped for present and future benefit.

A meeting of an international group concerned with issues related to the measurement of disability was convened in Delhi in December 2002. A sub group, mandated with the responsibility of discussing issues related to the assessment and measurement of impairment, generated a number of research questions. One of the issues raised was that of standards for surgery. It was suggested that surgeons and therapists have there are widely differing views on criteria for the variety of surgical interventions commonly offered to people with the secondary effects of leprosy. It was agreed that, if possible, it would be beneficial to publish standard assessment criteria for two principle reasons: (i) to assist inexperienced surgeons who may need authoritative guidance; and (ii) to have standardized procedures so that comparative studies may be conducted.

A method to gather information for guidance which is less compromised than that of an individual’s clinical experience in isolation is the Delphi method of consensus generation. A review of the method is included in an article elsewhere in this JOURNAL (Consensus Methods: A Bridge Between Clinical Reasoning and Clinical Research? See page 28 for this editorial).

It was agreed by the sub group that an attempt should be made to apply the method to address the issue of assessment criteria for a common procedure for the correction of foot drop (tibialis posterior transfer a.k.a. TPT).

A list of names of internationally recognized surgeons and therapists was gathered. Thirteen people were requested to consider participation in the process. Three declined but the remaining nine committed themselves to participation in, and the outcome of the Delphi Exercise. The participants, hereafter referred to as the “Delphi Team,” remained anonymous throughout the investigation to comply with the demands of the Delphi Exercise.

The Delphi Team comprised: Dr. J. W. Brandsma RPT, PhD, Consultant Physiotherapist, International Nepal Fellowship, Nepal; Dr. M. Ebenezer MBBS, D.Ortho., M.S(Ortho), Senior Specialist and Deputy Director, Schieffelin Leprosy Research and Training Center, Karigiri, India; Dr. R. Kazan, MD, Formerly Head of Surgery ALERT, Addis Ababa University, Ethiopia.

In 1981, Malaviya (2) compared Selvapandian’s surgical method with Srinivasan’s. (Malaviya reported follow-up of 78 cases from one to nine years and reported good results for either procedure in 70% of cases.) Malaviya emphasized the importance of post-operative physiotherapy as an important factor influencing outcome. When current protocols were reviewed for this study it was found that physiotherapy assessment criteria, and outcome indicators generally differed between institutions.

Bari, et al. (3) conducted circumtibial transfer of tibialis posterior and chose “heel toe gait restoration” and “active dorsiflexion” as indicators of success. Soares (4,5) compared circumtibial with interosseous methods of tendon transfer. The outcome he was primarily interested in was recurrent inversion deformity. From the review of current assessment protocols, it was found that the restoration of heel toe gait and dorsiflexion were common but inversion deformity was not. (In the final draft, the occurrence of inversion deformity is considered to represent a “failed” procedure).

A criterion that was noticeable by its absence in current assessment protocols was client satisfaction. Weber, et al. (6) studied 25 cases of TPT using levels of patient satisfaction as the outcome of interest. Finding that 18 were satisfied but 7 were not, Weber considered the procedure to be appropriate in a developing country (his study was undertaken in Pakistan).

The findings in all the studies cited above would have been strengthened if assessment methods had been standardized and a wider set of outcomes had been considered.

METHOD

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Some studies of the surgical procedure have been published. In 1981, Malaviya (2) compared Selvapandian’s surgical method with Srinivasan’s. (Malaviya reported follow-up of 78 cases from one to nine years and reported good results for either procedure in 70% of cases.) Malaviya emphasized the importance of post-operative physiotherapy as an important factor influencing outcome. When current protocols were reviewed for this study it was found that physiotherapy assessment criteria, and outcome indicators generally differed between institutions.

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The Sequence of developments in the Delphi Exercise:

Stage 1. An initial letter explaining the Delphi process and inviting participation was sent, by email, to potential Delphi members. The message had a request that, should they agree to participate, they should submit any contemporaneous TPT assessment forms known to them.

Stage 2. Collation of information from assessment forms.

Stage 3. Dispatch of collated criteria for rating by Team Members.

Stage 4. Scoring of results from rating exercise.

Stage 5. Dispatch of agreed criteria for further refinement by Team Members: i.e., contraindications, assessment indicators and expected outcomes.

Stage 6. Collation of results from refinement exercise.


Stage 8. Final adjustments to Gold Standard in accordance with feedback from Delphi Team.

Stage 9. Presentation of final product (Gold Standard) to the Delphi Team.


**Stages 1 and 2: Collation of Information.** Eight people submitted assessment forms from which information was collated by the coordinator. A list was compiled which included all the criteria contained in the various forms. On examining the assessment forms, it was apparent that the process of assessment progressed through stages with key components taking prominence at each stage. A total of 69 discrete criteria for screening and pre-operative assessment were identified in the assessment forms that had been submitted. These criteria applied to five essential stages of assessment, which were identified as: (i) Initial Screening; (ii) Pre-Physiotherapy—Psychosocial; (iii) Pre-Physiotherapy—Physical; (iv) Pre-Physiotherapy—Physical—Muscle Grading (MRC); and (v) Pre-Operative Screening.

From the assessment forms that were submitted only 16 of 69 criteria were common to 5 or more forms.

TPT assessment forms included post-operative assessments. The number of discrete criteria identified in post-operative assessments was 44, but only 3 criteria were found to be common to 5 or more of the forms submitted.

**Stage 3.** All discrete criteria were tabulated. The task, as explained to the Delphi Team, is given below (Note. Full tables available on-line from the ILA website, www.leprosy-ila.org. They may also be obtained from the author.)

(i) The tables contained lists of all the criteria collated from the assessment forms that were sent to the coordinator.

(ii) Eight people had submitted forms that were in use, at divers’ institutions, at the time of the exercise. Where 5 or more people submitted forms that contained the same criteria, such criteria were considered “essential.” These criteria were listed in the table but did not require any further consideration.

(iii) A column denoted “F” was included. The number related to the number of forms from different institutions where a particular criterion was already being used.

(iv) Each team member was required to rank every criterion except those that were already accepted as “essential.” The rank options were: Should be omitted, Not Useful, Neutral, Useful, and Essential.
Team members were informed that it would be assumed that personal details of patients (including hospital number, etc.) would be included in all assessments, along with the name of the person undertaking the assessment. It was also assumed that the general health of the patient will be assessed before consideration of surgery. Criteria relating to General Health, therefore, were not included either. Team members were reminded, however, that an aim of the exercise was to produce an assessment form that could be used with confidence by surgeons and therapists who may have limited or no experience with leprosy.

Stage 4. Nine people responded to the request to complete the task of ranking the criteria. Each rank was given a score. Descending negative scores were given where members had ranked a criterion as either “Not useful” or “Should be omitted.” If a criterion was ranked as “Neutral” it was scored as 0. Ascending positive scores were given where members had ranked a criterion as either “Useful” or “Essential.” With nine members contributing, the final score for each criterion represented the mean of the nine responses.

Criteria were judged as follows:
Score < 1. The indication was that the criterion is Not Acceptable and should be rejected.
Score > 1 < 2. The indication was that the criterion is Acceptable and should therefore be included for further consideration.
Score > 2 < 3. The indication was that the criterion is Useful and should therefore be included.
Score > 3. The indication was that the criterion is Essential.

Stage 5. All criteria meeting acceptance were tabulated and resubmitted to the Delphi Team for their information (please see Appendix 2). Two criteria were withdrawn because they caused confusion (gauged by comments from team members).

This stage of the exercise also required that the Delphi Team should again consider the criteria and state how the criteria should be used as indicators and contra-indicators for surgery. Post operative assessment criteria were also tabulated with key assessment times (according to the group vote). The Delphi Team was asked to consider the criteria for assessment and, to give a concise description of expected outcomes.

Stage 6. Contraindications for TPT surgery. Eight people submitted suggestions for contraindications. Where 4 or more people identified the same or similar contraindication, the suggestion was recorded as an “absolute contraindication.” Where fewer than 4 people submitted a suggestion, the contraindication was recorded as a “relative contraindication.”

Expected Post Operative Outcomes. While similar in meaning, the outcomes that were submitted were different in the way they were expressed. This was due mainly to the method, which at this stage was more open to personalized expression. To tabulate the expected outcomes, the most representative expression of an item was selected by the coordinator. The manner in which items were represented was also edited for clarity and conformity by the coordinator.

Stage 7. On receipt of the responses from stage 6, the assessment criteria, with contraindications, were again tabulated as were the expected outcomes. These tables were resubmitted to the Delphi Team as “the draft Gold Standard.” Assurance was given that should 4 or more people request changes to any one item in the tables, such requests would be implemented (this was because the wording had been edited by the coordinator and was therefore subject to his interpretation). It was reiterated, however, that the screening, assessment and outcome criteria per se were no longer negotiable (see Appendix 3).

At this stage, team members were made known to each other and the process was opened for discussion should the team wish to inter-relate, mindful that the objective of the exercise was to present a consensus on criteria that could be recommended as “Gold Standards” for TPT protocol. The aim was not to produce an actual protocol, but that the criteria should represent the key elements for prospective protocols (actual protocols will be institution-specific). Members were requested to consider the following options and then to indicate their choice to the coordinator: (i) Endorse the elements as they stood. (This was to be the preferred choice if members were satisfied with the contraindications, assessment criteria, and expected outcomes as given in the
draft “Gold Standard.” If at least 70% of the members agreed to endorse the criteria then the element would be presented as a “Gold Standard.”); (ii) Endorse the elements, but offer personal comments to augment them; (iii) Request delay of endorsement pending further discussion; (iv) Reject the elements.

**Stage 8.** Nine members responded to the request for refinement of the draft “Gold Standard.” Three members endorsed the elements as they stood. Six others had some criticism of, or sought clarification of different criteria, but no criterion had more than 2 requests for the removal or alteration of the criterion. None of the members expressed dissatisfaction with the outcome in its entirety.

In response to requests, the coordinator altered the wording of three elements that had consistently caused confusion.

**Stage 9.** The final draft of Gold Standard Criteria for TPT was circulated to all members with expressions of gratitude for their collaboration.

**Stage 10.** Papers drawn from the process and outcome of the exercise were written up for publication.

**RESULTS**

On the basis of consensus as described in Delphi methodology, a list of criteria was agreed on by a panel of recognized experts. The list includes:

- Criteria for the initial screening of potential candidates for Tibialis Posterior Transfer (foot drop correction). There are 10 essential criteria that should be considered when screening patients for suitability for surgery. Relative and absolute contraindications (or both) are given (Table 1).
- Pre-operative assessments. There are 20 criteria that should be considered essential aspects of examination before physiotherapy to prepare a person for surgery, and 5 essential criteria that should be satisfied during a surgeon’s pre-operative examination. For each of these examination criteria, either a relative or absolute contraindication (or both) are given (Table 2).
- Post-operative assessments. There are 17 essential criteria that should be examined post-operatively. Expected outcomes at periods after surgery are given (Table 3). Key post operative dates are fixed at: (i) one day after plaster of Paris removal; (ii) four weeks after post operative physiotherapy; (iii) between 3 to 6 months after post operative physiotherapy.

**DISCUSSION**

Sound empirical evidence is the most reliable basis on which clinical practice should be developed. There are situations, however, where the authority of individuals is validated by peer recognition of their experience and expertise. It is not sound to recommend practice based on the reputation of a single individual. Where a homogenous group of recognized experts can develop and endorse recommendations, however, the outcomes have internal validity and can be recommended. The validation of standardized assessment criteria will, furthermore, facilitate comparative studies which may yield empirical data that will further enhance the development of clinical practice.

Delphi is a method of consensus development among homogenous groups. While the design does control the negative effects of open group interaction it does also lose some of the positive effects of open interaction: e.g., idea generation. In this study, some members registered frustration with the isolation demanded by anonymity and seclusion and suggested that they would have preferred direct discussion and personal interaction. However, domination and control were avoided by the process thus allowing greater freedom of expression by some who may otherwise have perceived threat.

A Delphi Exercise is often protracted. The investigation presented here took 18 months to complete. The principal reason for this problem was the demands on the time of individuals in the Delphi Team. Without the immediacy and urgency dictated by the constraints of a physical meeting, members may be distracted from the task to attend to more pressing matters. However, an advantage of the method is that it can draw on the resources of individuals from diverse locations without the costs and inconvenience of physically assembling an international group.


<table>
<thead>
<tr>
<th>Criterion</th>
<th>Absolute Contraindication</th>
<th>Relative Contraindication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Physiotherapy—Determine Leprosy Status</td>
<td>Patient has not completed 3 months of PB leprosy treatment or 6 months MB leprosy treatment</td>
<td>Patient is currently taking MDT treatment</td>
</tr>
<tr>
<td>Duration of paralysis</td>
<td>Less than 6 months</td>
<td>(If uncertain of duration, query neurological testing and treatment given before proceeding)</td>
</tr>
<tr>
<td>Pre-Physiotherapy—Determine Neurological Status</td>
<td>Patient has presented with reaction, or acute neuritis within 6 months prior to consideration for surgery.</td>
<td>Patient has received steroid therapy within 3 months prior to consideration for surgery</td>
</tr>
<tr>
<td>Has the patient been on steroid treatment within the previous 6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Physiotherapy—Psycho Social Screening</td>
<td>Patient cannot commit to at least 1 month inactivity (work, family obligations or distance may prohibit)</td>
<td>Patient shows disinterest to do adequate pre surgical training and preparation</td>
</tr>
<tr>
<td>What is the patient’s occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does the patient understand:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Objectives of surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• That surgery will not change loss of nerve modalities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• That active collaboration will be required for successful outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does the patient understand and accept:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Time required (including pre and post operative physiotherapy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Time of reduced activity required before resuming normal activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can the patient demonstrate adequate self care</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has there been an assessment of the activities of daily living</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and the patients participation in social activities</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 2. Examination of TPT Candidates

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Absolute Contraindication</th>
<th>Relative Contraindication</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre Physiotherapy—Physical Examination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any signs (or history) of neurological bone disorganization</td>
<td>Active neurological bone disorganization (NBD)</td>
<td>History of NBD</td>
</tr>
<tr>
<td>Skin Condition</td>
<td>Ulceration, fungal infection or dermatitis on the foot to be operated on</td>
<td>Ulceration on any other body part</td>
</tr>
<tr>
<td>Any other eye hand or foot impairments</td>
<td>Joint Limitation / block restricting dorsiflexion (May be addressed prior to surgery)</td>
<td>Inability to use crutches</td>
</tr>
<tr>
<td>Is there any contracture of plantarflexor muscles</td>
<td>If the foot is fixed in either inversion or eversion</td>
<td></td>
</tr>
<tr>
<td>What is the position of the foot at rest (inverted or everted)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Define quality and range of motion at foot joints other than the ankle joint</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Define gait pattern</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Describe arch architecture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascertain whether patient can use crutches</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Describe the type of footwear that has been used and the duration of usage</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MRC Grading:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tib. Anterior</td>
<td>Less than MRC grade 4</td>
<td></td>
</tr>
<tr>
<td>Ext. Hal. Longus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ext. Dig. Longus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flex. Hal. Longus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flex. Dig. Longus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per. Longus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per. Brevis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tib. Posterior</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tendo Ach. / Gastochnemius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is there evidence of claw toes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre Operative Examination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin condition</td>
<td>Ulceration, fungal infection or dermatitis on the foot to be operated on</td>
<td>Ulceration on any other body part</td>
</tr>
<tr>
<td>Passive dorsiflexion / Knee in flexion / Knee in extension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active dorsiflexion / Knee in flexion / Knee in extension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active plantarflexion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive range of motion (ankle)</td>
<td>Exclude if &lt;10 deg dorsiflexion (unless caused by correctable contracture)</td>
<td></td>
</tr>
<tr>
<td>Criterion</td>
<td>Expected Outcomes and Considerations</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>--------------------------------------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 Day after POP removal</td>
<td>After 4 Weeks Physiotherapy</td>
</tr>
<tr>
<td>Position of foot at rest (eversion / inversion)</td>
<td>NA</td>
<td>Neutral to slight eversion</td>
</tr>
<tr>
<td>Active range of motion/Knee in flexion</td>
<td>NA</td>
<td>20 degrees dorsiflexion with recovering plantarflexion</td>
</tr>
<tr>
<td>Passive dorsiflexion / Knee in extension</td>
<td>20–25 degrees from ankle neutral</td>
<td>15 to 20 degrees from ankle joint neutral</td>
</tr>
<tr>
<td>Active dorsiflexion / Knee in extension</td>
<td>Partial (0–10 degrees)</td>
<td>10 to 20 degrees (minimum 10) from ankle joint neutral (90 degrees)</td>
</tr>
<tr>
<td>Gait pattern</td>
<td>NA</td>
<td>Able to bear full weight, heel to toe plantarflexion</td>
</tr>
<tr>
<td>Active Plantarflexion</td>
<td>NA</td>
<td>10–20 degrees from ankle joint neutral</td>
</tr>
<tr>
<td>Able to walk normally (short distance)</td>
<td>No walking</td>
<td>Normal walking</td>
</tr>
<tr>
<td>Able to squat</td>
<td>No squatting</td>
<td>Not to be encouraged</td>
</tr>
<tr>
<td>Arch architecture</td>
<td>Normal</td>
<td>Normal; i.e., should resemble pre op architecture</td>
</tr>
<tr>
<td>Inversion with dorsiflexion</td>
<td>If present the procedure is a failure</td>
<td>No</td>
</tr>
<tr>
<td>Eversion with dorsiflexion</td>
<td>No</td>
<td>Check foot after beginning weight bearing and intervene if needed</td>
</tr>
<tr>
<td>Signs of neurological bone disorganization</td>
<td>NA</td>
<td>Check and take needed intervention. Sign will indicate poor therapy</td>
</tr>
<tr>
<td>Signs of ulceration on lateral border</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Inversion deformity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suitability of Footwear/orthosis</td>
<td>NA</td>
<td>Appropriate tie up (or velcro) shoe with orthosis</td>
</tr>
</tbody>
</table>

*This item should be checked. It is not an outcome*
Control of coordinator bias can be an issue. It is imperative that the coordinator of the Delphi Team maintains complete impartiality and strives to ensure that all submissions are correctly interpreted and that all requests for clarification are conveyed. It is preferable that when a Delphi Exercise is commissioned, a coordinator is appointed who will not have a vested interest in the outcome of the process. Presenting ideas unambiguously and with sufficient clarity for all members to grasp is an essential and demanding task. For this reason simplicity and brevity are recommended.

Consensus does not imply unanimity. It is the product of negotiation and compromise and represents a general agreement. Not all the members of the Delphi Team endorsed all the criteria recorded (although only a very few criteria evoked criticism). The final outcome is a valid reflection of the corporate opinion of the experts who participated in the Delphi Exercise. Individual therapists and surgeons will add features that they may consider will give more detailed information on a case by case basis.

CONCLUSION

A consensus was reached on the essential criteria to be considered when surgeons and therapists are planning and executing Tibialis Posterior Transfer to address the problem of foot drop. That the exercise was necessary is supported by the observation that the pre- and post-operative protocols that were submitted at the start of the exercise differed greatly in the assessment criteria used.

The outcomes are presented with two recommendations: (i) best practice can be developed on the basis of the criteria suggested; and (ii) comparative studies of the TPT procedures will benefit from standardized protocol based on the recommendations of the Delphi Team.

REFERENCES

Leprosy is a chronic granulomatous disease characterized by hypopigmented and anesthetic skin lesions. Visible lesions occurring over the face, especially those situated over the malar region or over the eyelids, are known to be associated with lagophthalmos (3). Although the face has a rich sensory nerve supply, it has been shown that areas of anesthesia can exist in the face in leprosy patients (1, 4). Patients are often unaware of injuries that occur over these anesthetic areas. We report on one such patient who sustained an injury over the left upper eyelid and three years later the skin taken from that region during surgery for entropion disclosed histopathologically, a foreign body granuloma.

CASE REPORT

A 53-yr-old female presented with severe itching of the left eye due to a flaccid entropion and trichiasis. Skin and muscle excision over the left upper eyelid with deep sutures to correct the entropion was done and the excised skin sent for routine histopathological examination. The patient was diagnosed as having lepromatous leprosy 35 yrs ago. Her initial skin smears, done in 1965 for acid-fast bacilli (AFB) had an average bacterial index of 1.80+. She was treated with Dapsone monotherapy for 17 yrs followed by the Multidrug Therapy (MDT) recommended by the World Health Organization (WHO) for two years. From 1979 onwards her skin smears, done every year, had been negative for AFB. On examination, there was a glove and stocking anesthesia, both ulnar nerves were enlarged, and collapsed nose was present. The 5th toe of the right foot was lost. The right eye had a best corrected visual acuity of 6/24, madarosis, flaccid entropion, trichiasis, decreased corneal sensation, old keratic precipitates, non-reacting pupil, iris atrophy, and cataract. The left eye had a visual acuity of counting fingers at 1 meter, mild lagophthalmos, which on gentle closure of the lids did not expose the cornea, flaccid entropion, trichiasis, decreased corneal sensation, and vascularized corneal opacity.

Histopathology of the skin from the eyelid displayed granulomas composed of foreign body giant cells and histiocytes in the stroma (Fig. 1). When a polarizer was used to view the field, polarizing foreign particles were seen (Fig. 2). Acid-fast staining did not reveal any AFB. The patient had not volunteered any history of injury, but on questioning whether any injury had occurred, said that a piece of sugar cane had lodged in her upper lid when she was cutting it three years ago. There was no pain and the open lesion had healed. Sensation over the face was checked using three grades of Semmes Weinstein monofilaments (2) and disclosed large areas of anesthesia over the face including the lids of both eyes.

COMMENT

Detection of the foreign body granuloma by histopathology was accidental. It is likely that the fiber-like foreign bodies engulfed by the giant cells are the sugarcane fibers that had lodged in the upper eyelid.
three years ago. The significant aspect of the injury was that a considerable period of time had passed without the patient attaching any undue importance to it, but was highlighted because of the incidental histopathological finding and retrospective questioning.

Sensory loss over the limbs can lead to destruction of the extremities and this has led to the establishment of numerous prevention of disability (POD) programs in places where leprosy is still endemic. These programs hardly ever take into account or evaluate loss of sensation over the face.

Fig. 1. Photomicrograph showing granulomas composed of foreign body giant cells and histiocytes in the stroma of eyelid skin. (H&E, ×200.)

Fig. 2. Polarized photomicrograph showing foreign bodies in the stroma. (H&E, ×200.)
This report emphasizes the fact that sensory loss over the face could also result in injuries that go unnoticed and uncared for by the patient. In this case, the injury was trivial but if the injury had occurred on an insensitive cornea, a fungal corneal ulcer destroying the eye would not only have been a possibility but, had it occurred, could have led to blindness and added to the suffering of a group of people already overwhelmingly disadvantaged.

REFERENCES
A century ago, most of those who worked on leprosy did so in near isolation, scientifically and geographically. Their situation was chaotic, intellectually and otherwise: leprosy had a confusing diversity of clinical manifestations, classifications, and complications. It was incurable, and caused enormous upheaval in the families and communities where it occurred. Most workers were missionaries, and there was little financial support for research. The overall situation improved after dapsone became available to cure the infection, but leprosy still did not attract many medical scientists.

This situation changed dramatically in the 1960s, with an extraordinary coincidence of scientific thinking and discovery that led to a "golden age" of leprosy research. In a chapter on "The Immunopathologic Spectrum of Leprosy" (1964), Olaf Skinsnes presented the first full formulation of the concept we now consider basic to the understanding of leprosy, i.e., that the diversity of clinical, pathological, and microbiological findings in leprosy are a result of varying degrees of cellular immunity to *Mycobacterium leprae* in different patients (6). Scarcely two years later, Drs. Ridley and Jopling published their practical classification system that was congruent with this theoretical foundation (5). Based on clinical and histopathologic findings, this classification system enabled physician investigators around the world to classify patients according to a common standard. The combination of a well-grounded theory and a practical method of universal classification gave new impetus to research.

Meanwhile, during the 1960s immunologists identified the distinction between T cells and B cells, and recognized their respective roles in cellular and humoral immunity (e.g., references 3 and 4). Scientists rapidly developed an entirely new set of tools, and simultaneously discovered leprosy as a challenging human disease that appeared to be an ideal model in which to examine theories and methods related to cellular immunity in man.

The convergence of these developments prompted an extraordinary burst of research effort and publications that increased in a linear fashion from a nadir of 3 papers in 1962 to a maximum of 172 papers in 1989 (The Figure). This approach to assess the extent of scientific effort expended per year is crude, and may miss some publications. It does, nevertheless, offer a reasonable estimate of the trend with respect to the level of research activity as reflected by publication in the scientific literature. A total of over 2000 medical and scientific publications indexed on "leprosy AND immunology" appeared during this period of time.

And then, around 1989–90, the bottom appeared to fall out of this effort. The number of papers published annually on the immunology of leprosy began a decline that is as precipitous as its rise had been only a decade before (The Figure). At the current rate, we can expect that around 2010–11 there will once again be only 3 papers published on the immunology of leprosy.

What happened? Did the ability to cure infection with *M. leprae* bring an end to the inquiry? Were the compelling questions concerning human immunity answered? The answer to these questions is "no."

Even after effective monotherapy with dapsone was available, and additional effective agents were added to the treatment regimen, medical scientists were emphati-
cally agreed that it was imperative to understand the underlying mechanisms of this disease. The earnest introductions to hundreds of papers published from the 1960s through the 1980s brim with the conviction that leprosy was not only a major problem in the world, but that an understanding of its immunological characteristics would unlock profoundly important insights into this and other diseases. An unsuspecting observer might think that around 1990 the important, basic questions about leprosy had suddenly and decisively been answered; the mechanisms underlying the remarkable spectrum of leprosy must have been discovered, and immunotherapies and vaccines developed, and this scourge had been eliminated.

Among the developments during this period of time were new global health problems, especially HIV/AIDS, and a renewed concern about tuberculosis. These competing imperatives, however, do not obviate the oft-repeated assessment that leprosy remains an extraordinary scientific challenge that will yield important lessons for other diseases, as well. Another important factor was the inauguration in 1991 of the World Health Organization campaign to eliminate leprosy as a public health problem by the year 2000. The elimination has not happened, however, and sound, scientific epidemiological evidence and models clearly indicate that it will not happen anytime soon with only the methods of diagnosis and treatment now available (1, 2). Research into the underlying immunological mechanisms of this infection, however, has nearly been eliminated, as evidenced by the decline in publications.

What were the basic questions in leprosy that scientists of the 1960s, 70s, and 80s found so compelling? The proceedings of several ILA Congresses and workshops from the 1960s to the present, and the reports of WHO committees and advisory groups in the same half century, repeatedly asserted the high priority of the following basic research questions:

1. What is the mechanism of transmission of *M. leprae*?
2. Why is *M. leprae* an obligate intracellular parasite? What is this organism lacking that it cannot be cultivated?
3. What is the mechanism underlying the unique spectrum of cellular immune responses in leprosy, and the selective nonresponsiveness of polar lepromatous patients?
4. What is the mechanism of Type 1 reactions?
5. What is the mechanism of Type 2 reactions?
6. What is the mechanism of nerve injury?

All of these questions remain unanswered today, and the last 4 of 6 in this list are closely related to the immune response to *M. leprae*. However, the perception that the elimination of leprosy is imminent has undoubtedly discouraged many scientists and funding sources from pursuing it further. The unfortunate experience of premature de-emphasis on research in such infectious diseases as tuberculosis and malaria, however, suggest that with a disease as slow but persistent as leprosy, continued effort to understand the underlying mechanisms of disease is essential to the quest for genuine success in conquering it.

—DMS

REFERENCES

COMMENTARY

Introductory note:
As the JOURNAL embarks on a policy encouraging papers of medical and scientific value from disciplines sometimes unfamiliar to us, such as the social sciences, readers may find that some of the methods used to collect or analyze data are also unfamiliar. In this issue, we present an important report concerning evaluation of techniques for surgical reconstruction (Page 13). A consensus method was used to reach the conclusions in this report, based on responses from several highly experienced individuals. Dr. Hugh Cross has kindly consented to provide the following background information regarding the consensus method used in this study. Ed.

Consensus Methods: A Bridge Between Clinical Reasoning and Clinical Research?

ABSTRACT
Evidence-Based Practice does head the “hierarchy of evidence” upon which developments in clinical practice should be based. There are, however, situations where evidence is either unavailable, unclear, or results between studies are at variance. Consensus is a reliable contingency, and approaches to reaching consensus have acceptable construct validity (Nominal Group Technique, Delphi, and Consensus Development Conference).

Consensus is reached when: (i) the method of investigation tightly controls communication to reduce the obscuring “noise” of divergent discussion; (ii) statistical measures of agreement or dissent screen out the bias that would otherwise be produced by the dictate of vociferous minorities or coalitions that may represent vested interests; (iii) all participants contribute equally to the product of the investigation.

RÉSUMÉ
Le concept de la pratique médicale basée sur des données établies (dénommée « Evidence-Based Practice ») permet réellement d’établir une « hiérarchie des preuves », à partir de laquelle des développements utiles pour la pratique médicale cliniques peuvent être déployés. Il y a cependant des situations où les données cliniques ne permettent pas de clairement étayer une hypothèse médicale ou soutenir une observation; ou bien les résultats observés d’une étude à une autre présentent une variation importante. Le Consensus est alors une méthode robuste dans de tels cas, et la plupart des approches pour atteindre un consensus, telle que par exemple la Technique du Groupe Désigné, la Méthode de Delphi et la Conférence de Développement du Consensus, présente une démarche bien construite et de validité acceptable.

Un consensus est atteint lorsque: (i) la méthode d’investigation contrôle efficacement la communication, afin de réduire le brou de fond parasite et inutile des discussions divergentes; (ii) des mesures statistiques d’accord ou de désaccord filtrent les biais qui pourraient être produits par le dictat de minorités véhémentes ou bien de coalitions qui pourraient avoir des intérêts cachés; et (iii) tous les participants contribuent de façon équilibrée au produit de l’investigation.

RESUMEN
La Práctica Basada en la Evidencia reconoce la “jerarquía de la evidencia” como la base sobre la cual deben apoyarse los avances en la práctica clínica. Hay, sin embargo, situaciones donde la evidencia no es accesible, es poco clara, o hay variación en los resultados obtenidos. El consenso es una contingencia confiable y los intentos de alcanzar el consenso tienen una aceptable validez constructiva (Nominal Group Technique, Delphi, and Consensus Development Conference).

El consenso se alcanza cuando: (i) el método de investigación controla estrechamente la comunicación para reducir el ruido de la discusión divergente, (ii) las mediciones estadísticas de concordancia o de no concordancia toman en cuenta todas las tendencias o inclinaciones ya que de otra manera las opiniones de minorías o coaliciones vociferas podrían ser dominantes, (iii) todos los participantes contribuyen igualmente al producto de la investigación.
Consensus is reached when: (i) the method of investigation tightly controls communication to reduce the obscuring “noise” of divergent discussion; (ii) statistical measures of agreement or dissent screen out the bias that would otherwise be produced by the dictate of vociferous minorities or coalitions that may represent vested interests; (iii) all participants contribute equally to the product of the investigation.

With the publication of the Report of the International Leprosy Association Technical Forum (1) it has become broadly accepted that, wherever possible, further decisions on any proposed developments in clinical practice should be evidence-based. Proponents of evidence-based practice (EBP) suggest that decisions based on the empirical paradigm of science are less likely to be compromised by the unpredictable elements of subjectivity that are probably inextricable from clinical reasoning.

There are, however, circumstances where EBP does not provide answers for those who face problems of decision-making. For situations where there is already a plethora of confusing information, statistical methods such as meta-analysis are now in common use. Where published information is inadequate, non-existent, or contradictory, however, consensus methods provide a means of synthesizing the insights of experts to create a product that decision-makers can use with relative confidence.

Consensus as a valid construct has been supported through exercises in the fields of social science with the result that three objective methods of consensus building and reporting are now in common use: The Delphi Investigation, The Nominal Group Technique (NGT), and Consensus Development Conference. Each shares the common objective of synthesizing judgments when a state of uncertainty exists, but whereas Delphi and NGT are appropriate for smaller scale investigations, the Consensus Development Conference was designed to resolve conflicting opinions and contentious issues that impact on health policy at national or international levels. (Henceforth, this communication will only consider Delphi and Nominal Group Technique as these lie within the experience of the author.)

The central question of consensus reliability was investigated in early studies by Delbecq and Van de Ven (2) who ascertained that judgmental accuracy may be achieved where the following features are encapsulated in the method of investigation: (i) individuals make independent judgments; (ii) individual judgments are be expressed through mathematical rank-ordering and/or rating of items; the mean value of independent judgments are accepted as indicating group decision; re-voting should follow discussion of the mean values.

More recent studies have shown that an indication of the distribution or dispersal of participants’ judgments, not just the measure of central tendency, is more appropriate. In general, the median and the inter-quartile range are more robust than the mean and standard deviation. Further thematic content analysis of comments and discussion can also enhance the quality of outcomes. (2,3,4,5)

The Nominal Group Technique. An essential feature that characterizes a “group” is verbal communication. The reason that the term “Nominal” was adopted is that it denotes group situations in which non-verbal communication is permissible (the group is therefore, by definition only nominally a group). Early researchers applied the term rigidly, and no verbal communication was permitted. However, most contemporary NGT investigations are essentially a development of the approach as both verbal and non-verbal stages are incorporated. Research has shown that by allowing the combination of verbal and non-verbal stages, the optimal benefit from a NGT investigation can be achieved.

Delbecq, et al. (6) approached the issue of consensus development from psycho-social studies of decision making processes. Their first considerations were the various effects of normative behavior on individuals in groups and on a group as an entity. They also considered studies of alternative processes on the performance of group decision making in terms of the quantity and quality of ideas generated; the affectional (emotional and expressive) overtones of interaction; and the nature of facilitative and inhibitive influences on creative problem solving.

An objective of the NGT is that normative behavior (which basically favors the performance of dominant or aggressive characters) will be controlled by nonconformance tactics so that performance and outcome are maximized, while hidden agendas and negative group dynamics are sup-
pressed. The NGT aims to draw out minority opinions and promote the tolerance of conflicting ideas.

Effective creative, or judgmental, problem solving passes through two essential phases simply defined as the “fact finding phase” and the “evaluation phase.” NGT includes processes that encourage deep consideration of problems and the enhancement of idea generation (fact finding), the clarification and synthesis of ideas (evaluation), and extends to all participants, an equal opportunity to contribute to the group product and to influence the direction of the decision outcome.

“The fact finding phase.” Van de Ven and Delbecq (7) had reported the human tendency to seek solutions before a question or problem has been adequately grasped (an effect exacerbated by relative degrees of heightened anxiety over the nature of the topic or the perceived situational threat. This tendency leads to poor quality decisions). They also observed that where verbal communication is the method of idea generation, there is the possibility of “focus effect.” “Focus effect” denotes a situation where group members are distracted and a single train of thought may be given inappropriate status. As a consequence, time is monopolized without the compensation of enhanced productivity. Van de Ven and Delbecq (7) also found that where group members are denied the opportunity for private reflection on independent thoughts, ideas were expressed as generalizations leaving individuals reluctant to be specific. The NGT method was developed to address these confounding effects as well.

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Equality of participation. Selection bias and the definition of expertise are the most commonly cited flaws in consensus investigations generally (2, 9, 10, 11). The choice of participants is a salient consideration. Commitment to the process requires an internal acknowledgment of participant homogeneity and an external recognition of the expertise represented in the group. A tenet of The Nominal Group technique is that idea quality and not presenter status is predominant.

The Delphi investigation. Since its conception in the 1950s when it was used by the Rand Corporation for use in defense related problems (12), Delphi has been applied extensively to clarify issues that have required sharper definition. The method is usually adjusted to suit the requirements of individual applications (it has been widely applied among health disciplines for investigations as diverse as the determination of diagnoses, through policy development to ascertaining criteria for professional competence). Essentially the Delphi method is supported on the same theoretical basis as the NGT but the interaction, controlled by a central facilitator, is conducted by mail. It has the advantage of including participants who are separated geographically. The isolated and wholly anonymous situations in which participants process and respond to information, without the pressure of immediate response, does result in a broader array of high quality ideas. However the positive affects of the group interaction component in the NGT are also lost. It is for this reason that some consider the Delphi method to be inferior to NGT.

Delbecq, et al. (6) were instrumental in the early developments of the Delphi Method. They suggested that for a Delphi investigation, sample size should be dictated by the homogeneity of the group and the nature of the investigation. A large sample is necessary if the principal reason for conducting the procedure is to develop awareness within a group, or where diverse reference groups are involved. Where the
desired outcome is to validate opinions based on experience, they suggested that a group of ten to fifteen participants is adequate for a homogenous group. Increasing the size of a homogenous group beyond 30 will not result in more information and only increases administration difficulties.

Methodological details vary according to the requirements of individual Delphi projects. The general approach has been that sequential, structured questionnaires have been used for participants to rank, or rate, responses to indicate his/her priorities related to the topics of interest. On receipt of returned questionnaires, information is collated and analyzed before being redistributed for further refinement and for final comments.

Information, in the form of a statistical analysis, should be dispatched to participants at subsequent rounds of the procedure. Feedback analysis should include the frequency with which participants selected answers, with the mean and/or median and one measure of dispersion. Individuals are asked to reconsider the scores applied previously (in the light of aggregated responses of all members) and, in this manner, consensus is generated.

As with NGT, the selection and definition of experts is cited as being the most potentially confounding effect on a Delphi outcome. Panelists are usually (though not exclusively) recruited by merit of an intimate academic or experiential association with the topic under investigation. Acknowledged expertise or influence may validate a choice of participants; however, Delbecq, et al. (6) suggested that such attributes per se are insufficient for the inclusion of participants. They cautioned that commitment to the investigation, motivation to comply with the demands of procedure and the acceptance of the consensus (even though it may be at variance with personal inclination) are fundamental. The “nature of the respondent panel, the obligations of participants, the length of time the Delphi process will take and the information that will be shared among participants” are variables likely to effect the co-operation of invited individuals and should be declared at the initial stages of recruitment.

Criticisms. Some have considered consensus methods from an epistemological perspective and cautioned against “overselling” the methods (2). A principal concern is that there is a risk that observers may place too great a reliance on consensus outcomes than may be warranted. This is a valid concern because consensus methods are used to generate quantitative estimates which could be misconstrued in some cultures as representing a “correct” answer. Consensus, is of course not synonymous with being invariably correct, but a responsibility lies with investigators to present outcomes with due consideration for the target readership. Sackman (9) represents the views of some who argue that Delphi outcomes represent a “forced” consensus that is further compromised because participants are not allowed to discuss issues.

Murphy, et al. (3) conducted an extensive review of published research using consensus methods. A result of their endeavor is that a guide has been published that should be considered by those considering the use of either method. One of the objectives of their survey was “To identify the factors that affect the decisions that emerge from consensus development methods.” Their study identified recurring methodological issues which they sought to isolate and address. What their study has shown is that the methodological issues that have caused the most controversy can be addressed.

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Might this compound or compound class be of value in the treatment of leprosy? The broad spectrum activity of R207910 against a range of mycobacteria (but not of non-mycobacterial species) suggests that it will likely be active against the leprosy bacillus as well. In addition, the M. tuberculosis and M. leprae ATPase proteins share 92.6% identity, again suggesting that the latter may well be highly susceptible. The long half-life and ability to shorten the treatment duration required for organ sterilization in M. tuberculosis-infected mice suggests that, should the spectrum of activity extend to M. leprae, this compound (or compound class) may help shorten the duration of leprosy treatment as well. Combinations containing R207910, a rifamycin and a fluoroquinolone—all of which appear to be both bactericidal and to have the ability to eliminate some percentage of persistent mycobacteria—may dramatically shorten treatment duration in both tuberculosis and leprosy, even in patients with a relatively high bacterial loads.

Of course much of this speculation regarding leprosy can be put to rest by a few well designed in vitro and in vivo experiments with M. leprae. While a phase I clinical trial has looked promising, the ultimate clinical utility in tuberculosis and in leprosy and other mycobacterioses can only be determined following phase II/III studies in these diseases.

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Consensus Methods: A Bridge Between Clinical Reasoning and Clinical Research?

ABSTRACT

Evidence-Based Practice does head the “hierarchy of evidence” upon which developments in clinical practice should be based. There are, however, situations where evidence is either unavailable, unclear, or results between studies are at variance. Consensus is a reliable contingency, and approaches to reaching consensus have acceptable construct validity (Nominal Group Technique, Delphi, and Consensus Development Conference).

Consensus is reached when: (i) the method of investigation tightly controls communication to reduce the obscuring “noise” of divergent discussion; (ii) statistical measures of agreement or dissent screen out the bias that would otherwise be produced by the dictate of vociferous minorities or coalitions that may represent vested interests; (iii) all participants contribute equally to the product of the investigation.

RÉSUMÉ

Le concept de la pratique médicale basée sur des données établies (dénommée « Evidence-Based Practice ») permet réellement d’établir une « hiérarchie des preuves », à partir de laquelle des développements utiles pour la pratique médicale cliniques peuvent être déployés. Il y a cependant des situations où les données cliniques ne permettent pas de clairement établir une hypothèse médicale ou soutenir une observation; ou bien les résultats observés d’une étude à une autre présentent une variation importante. Le Consensus est alors une méthode robuste dans de tels cas, et la plupart des approches pour atteindre un consensus, telle que par exemple la Technique du Groupe Désigné, la Méthode de Delphi et la Conférence de Développement du Consensus, présente une démarche bien construite et de validité acceptable.

Un consensus est atteint lorsque: (i) la méthode d’investigation contrôle efficacement la communication, afin de réduire le bruit de fond parasite et inutile des discussions divergentes; (ii) des mesures statistiques d’accord ou de désaccord filtrent les biais qui pourraient être produits par le dictat de minorités vives ou bien de coalitions qui pourraient avoir des intérêts cachés; et (iii) tous les participants contribuent de façon équilibrée au produit de l’investigation.

RESUMEN

La Práctica Basada en la Evidencia reconoce la “jerarquía de la evidencia” como la base sobre la cual deben apoyarse los avances en la práctica clínica. Hay, sin embargo, situaciones donde la evidencia no es accesible, es poco clara, o hay variación en los resultados obtenidos. El consenso es una contingencia confiable y los intentos de alcanzar el consenso tienen una aceptable validez constructiva (Nominal Group Technique, Delphi, and Consensus Development Conference).

El consenso se alcanza cuando: (i) el método de investigación controla estrechamente la comunicación para reducir el ruido de la discusión divergente, (ii) las mediciones estadísticas de concordancia o de no concordancia toman en cuenta todas las tendencias o inclinaciones ya que de otra manera las opiniones de minorías o coaliciones vocieras podrían ser dominantes, (iii) todos los participantes contribuyen igualmente al producto de la investigación.
Consensus is reached when: (i) the method of investigation tightly controls communication to reduce the obscuring “noise” of divergent discussion; (ii) statistical measures of agreement or dissent screen out the bias that would otherwise be produced by the dictate of vociferous minorities or coalitions that may represent vested interests; (iii) all participants contribute equally to the product of the investigation.

With the publication of the Report of the International Leprosy Association Technical Forum (1) it has become broadly accepted that, wherever possible, further decisions on any proposed developments in clinical practice should be evidence-based. Proponents of evidence-based practice (EBP) suggest that decisions based on the empirical paradigm of science are less likely to be compromised by the unpredictable elements of subjectivity that are probably inextricable from clinical reasoning.

There are, however, circumstances where EBP does not provide answers for those who face problems of decision-making. For situations where there is already a plethora of confusing information, statistical methods such as meta-analysis are now in common use. Where published information is inadequate, non-existent, or contradictory, however, consensus methods provide a means of synthesizing the insights of experts to create a product that decision-makers can use with relative confidence.

Consensus as a valid construct has been supported through exercises in the fields of social science with the result that three objective methods of consensus building and reporting are now in common use: The Delphi Investigation, The Nominal Group Technique (NGT), and Consensus Development Conference. Each shares the common objective of synthesizing judgments when a state of uncertainty exists, but whereas Delphi and NGT are appropriate for smaller scale investigations, the Consensus Development Conference was designed to resolve conflicting opinions and contentious issues that impact on health policy at national or international levels. (Henceforth, this communication will only consider Delphi and Nominal Group Technique as these lie within the experience of the author.)

The Nominal Group Technique. An essential feature that characterizes a “group” is verbal communication. The reason that the term “Nominal” was adopted is that it denotes group situations in which non-verbal communication is permissible (the group is therefore, by definition only nominally a group). Early researchers applied the term rigidly, and no verbal communication was permitted. However, most contemporary NGT investigations are essentially a development of the approach as both verbal and non-verbal stages are incorporated. Research has shown that by allowing the combination of verbal and non-verbal stages, the optimal benefit from a NGT investigation can be achieved.

Delbecq, et al. (6) approached the issue of consensus development from psycho-social studies of decision making processes. Their first considerations were the various effects of normative behavior on individuals in groups and on a group as an entity. They also considered studies of alternative processes on the performance of group decision making in terms of the quantity and quality of ideas generated; the affectional (emotional and expressive) overtones of interaction; and the nature of facilitative and inhibitive influences on creative problem solving.

An objective of the NGT is that normative behavior (which basically favors the performance of dominant or aggressive characters) will be controlled by nonconformance tactics so that performance and outcome are maximized, while hidden agendas and negative group dynamics are sup-
pressed. The NGT aims to draw out minority opinions and promote the tolerance of conflicting ideas.

Effective creative, or judgmental, problem solving passes through two essential phases simply defined as the “fact finding phase” and the “evaluation phase.” NGT includes processes that encourage deep consideration of problems and the enhancement of idea generation (fact finding), the clarification and synthesis of ideas (evaluation), and extends to all participants, an equal opportunity to contribute to the group product and to influence the direction of the decision outcome.

“The fact finding phase.” Van de Ven and Delbecq (7) had reported the human tendency to seek solutions before a question or problem has been adequately grasped (an effect exacerbated by relative degrees of heightened anxiety over the nature of the topic or the perceived situational threat. This tendency leads to poor quality decisions). They also observed that where verbal communication is the method of idea generation, there is the possibility of “focus effect.” “Focus effect” denotes a situation where group members are distracted and a single train of thought may be given inappropriate status. As a consequence, time is monopolized without the compensation of enhanced productivity. Van de Ven and Delbecq (7) also found that where group members are denied the opportunity for private reflection on independent thoughts, ideas were expressed as generalizations leaving individuals reluctant to be specific. The NGT method was developed to address these confounding effects as well.

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Equality of participation. Selection bias and the definition of expertise are the most commonly cited flaws in consensus investigations generally (2, 9, 10, 11). The choice of participants is a salient consideration. Commitment to the process requires an internal acknowledgment of participant homogeneity and an external recognition of the expertise represented in the group. A tenet of The Nominal Group technique is that idea quality and not presenter status is predominant.

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REFERENCES
CORRESPONDENCE

This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of the 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.

Some Considerations on the Origin of Type 1 Reactions in Leprosy

TO THE EDITOR:

The World Health Organization (WHO) suggests the use of corticosteroids to differentiate a relapse from a reaction in cases in which new lesions appear after the completion of treatment with multi-drug therapy (MDT) (1). If lesions improve, it is a case of type 1 reaction that must be treated only with such drugs. This immunological phenomenon would not have significance other than the fact of being a response to remaining Mycobacterium leprae antigens that would be exposed to the host defenses. If a patient keeps having reactional episodes after anti-leprosy treatment, the anti-inflammatory therapy should be continued.

Nevertheless, I believe that reactional episodes may result from multiplication of bacilli that were not destroyed by treatment. If this hypothesis could be demonstrated, these reactions would be considered relapses, and that would be a reason for the WHO’s statistics on relapses to be changed.

In general, type 1 reactions occur in pre-existing lesions that may appear as hypochromic macules with sensory changes, sensation or well constituted borderline or tuberculoid lesions with chronic evolution. These reactions are often times exuberant and occur before, during, or even after release from treatment (1).

All these reactions are presented with the same clinical characteristics. The bacilloscopy may be negative or positive, and if positive, bacilli may show degeneration in reactional episodes occurring before treatment, as well as during treatment.

In the pre-sulphone era, the authors carefully observed the natural history of some reactional cases and reported tuberculoid patients with certain reactions in which bacilli and lesions disappeared spontaneously. After some time or even years of quiescence, reactions reappeared with lesions and bacilli (1, 5, 6). These observations suggest that the M. leprae may remain for long periods in a state of metabolic inactivity, inaccessible to organic defenses (possibly as persisters). At a certain moment, maybe because of intercurrent diseases or other immunological changes, bacilli start to multiply again, initiating a new reactional episode.

If that happened in the past, it may also happen today, i.e., the bacilli can remain as persisters and not be destroyed by the immune defenses or treatment. The cell mediated type 1 reaction may somehow be related to multiplication of bacilli. The degenerated aspect of the bacilli may result from the multiplication of bacilli and their exposure to the effects of drugs that are being used, or to the immune defenses. The microorganisms are destroyed and release antigens that give rise to a hypersensitivity reaction (type 1 reaction). In reactions occurring after treatment, if the number of bacilli is low, the patients become cured because the body defenses destroy the bacilli. If there are many bacilli and the organic defenses are unable to control the infection, there will be new reactions and
risk of nerve involvement and development of disabilities.

Recently, Shetty, et al. (4) studied 25 cases of borderline-tuberculoid leprosy that presented with new lesions from 1 to 13 years after being released from treatment. Viable bacilli were found in the footpad of inoculated mice in 48% of the biopsies of those patients. Remarkably, the incidence of viable bacilli was higher (58%) in those patients whose histopathology showed evidences of reversal reaction.

Waters (7) commentary on Shetty’s work, referred to his own patient with a tuberculoid lesion on the face that appeared 40 years after the patient had been apparently cured, and admitted that the authors presented evidence that viable bacilli can cause relapse in borderline-tuberculoid leprosy, and that these relapses may be associated with reversal reactions.

I also studied a patient similar to Waters (7). She was a patient who presented with extensive erythematous-pink flat lesions on the trunk and extremities, with a negative bacilloscopic index (BI), which disappeared after 2 years of treatment with chaulmoogra oil. More than 40 years later she presented a reactional episode with large erythematous plaques on the entire skin, with a positive BI (++++) of the lesions, during an unbalanced diabetes mellitus.

These observations reinforce my interpretation of type 1 reactions, i.e., they are the result of multiplication of persisters.

There are no proofs of this hypothesis being true, but on the other hand, there is nothing showing it to be wrong.

I think the WHO should look at type 1 reactions more carefully during the evaluation of MDT results.

TO THE EDITOR:

It was interesting to read the article “Neuropathic pain in leprosy patients” by Stump, et al. (3). They have noted that a fair number of patients continue to suffer from neuropathic pain in leprosy. This probably is due to the treatment cut off point of 6 months or 12 months, depending upon the type of leprosy. Some patients do continue to complain of paresthesia even long after the activity is subsided. The series of Stump, et al. includes cases that were still on treatment.

The clinical activity takes fairly longer than the bacteriological cure. The World Health Organization regimens are meant to “kill” the maximum number of germs in shortest possible time. The body has to take care of the scavenging and it might suffer in the process. The process of nerve regeneration further complicates the issue, and if irritants are present, paresthesia develops. Moreover, the compressing elements con-
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continued to persist and are “assisted” by intraneural and perineural fibrosis. As a consequence, a “neuroma in continuity” develops and the pain continues.

Mishra, et al. (1, 2), while reporting their observations on development of leprosy lesions, noted that at least some of the lesions start as a vague dysthesia, meaning that positive phenomena occurs before a negative phenomena (sensory loss). “Painless” nerve damage has been glorified as silent neuropathy. It is very likely that those nerves have whispered before destruction, which patients were not able to hear due to the faintness of the sound or their preoccupation with other things. Logically, pain (including paresthesia and dysesthesia is a part of neural affection its intensity may vary depending upon the type of affection and the speed of damage.

Probably, both neuropathic pain and inflammatory pain exist together in leprosy. Even in acute neuritis, the pain is more than what is expected in pain of a purely nociceptive nature. That is probably the reason that many times acute neuritis is referred to as acute painful neuritis. The contribution from inflammation and neuropathy may vary from patient to patient and from time to time. The paresthesia complained of is usually of an annoying type, and with the advancing age of patients many other discomforts are added to it. It will be interesting to relate the paresthesia with disease activity in “cured” patients but, on the other hand, it also scares me. Any suggestion or discussion about pain might exacerbate the problems because patients are relatively unstable emotionally and tend to develop dependence because of peculiar psychosocial effects of the disease.

Mild paresthesia can be managed with suggestions and counseling, whereas disabling paresthesia needs drugs in addition. But before all that can be formulated and put into practice, treatment of leprosy has to be modified from community approach to individualized approach.

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Recently, Shetty, et al. (4) studied 25 cases of borderline-tuberculoid leprosy that presented with new lesions from 1 to 13 years after being released from treatment. Viable bacilli were found in the footpad of inoculated mice in 48% of the biopsies of those patients. Remarkably, the incidence of viable bacilli was higher (58%) in those patients whose histopathology showed evidences of reversal reaction.

Waters (7) commentary on Shetty’s work, referred to his own patient with a tuberculoid lesion on the face that appeared 40 years after the patient had been apparently cured, and admitted that the authors presented evidence that viable bacilli can cause relapse in borderline-tuberculoid leprosy, and that these relapses may be associated with reversal reactions.

I also studied a patient similar to Waters (2). She was a patient who presented with extensive erythemato-hypochromic flat lesions on the trunk and extremities, with a negative bacilloscopic index (BI), which disappeared after 2 years of treatment with chaulmoogra oil. More than 40 years later she presented a reactional episode with large erythematous plaques on the entire skin, with a positive BI (++++) of the lesions, during an unbalanced diabetes mellitus.

These observations reinforce my interpretation of type 1 reactions, i.e., they are the result of multiplication of persisters. There are no proofs of this hypothesis being true, but on the other hand, there is nothing showing it to be wrong.

I think the WHO should look at type 1 reactions more carefully during the evaluation of MDT results.

—D.V.A. Opromolla

Division of Training and Research Instituto Lauro de Souza Lima
Address: P.O. Box 3021. Bauru. SP. Brazil. 17034-970

Dr. Opromolla passed away while this issue was in production. The JOURNAL extends its condolences to the family of this long-time professional in the leprosy field. Dr. Opromolla’s obituary will appear in the June issue of the JOURNAL.

REFERENCES

Neuropathic Pain in Leprosy Patients

TO THE EDITOR:

It was interesting to read the article “Neuropathic pain in leprosy patients” by Stump, et al. (5). They have noted that a fair number of patients continue to suffer from neuropathic pain in leprosy. This probably is due to the treatment cut off point of 6 months or 12 months, depending upon the type of leprosy. Some patients do continue to complain of paresthesia even long after the activity is subsided. The series of Stump, et al. includes cases that were still on treatment.

The clinical activity takes fairly longer than the bacteriological cure. The World Health Organization regimens are meant to “kill” the maximum number of germs in
Correspondence

shortest possible time. The body has to take care of the scavenging and it might suffer in the process. The process of nerve regeneration further complicates the issue, and if irritants are present, paresthesia develops. Moreover, the compressing elements continue to persist and are “assisted” by intraneural and perineural fibrosis. As a consequence, a “neuroma in continuity” develops and the pain continues.

Mishra, et al. (1,2), while reporting their observations on development of leprosy lesions, noted that at least some of the lesions start as a vague dysthesia, meaning that positive phenomena occurs before a negative phenomena (sensory loss). “Painless” nerve damage has been glorified as silent neuropathy. It is very likely that those nerves have whispered before destruction, which patients were not able to hear due to the faintness of the sound or their preoccupation with other things. Logically, pain (including paresthesia and dysthesia is a part of neural affection its intensity may vary depending upon the type of affection and the speed of damage.

Probably, both neuropathic pain and inflammatory pain exist together in leprosy. Even in acute neuritis, the pain is more than what is expected in pain of a purely nociceptive nature. That is probably the reason that many times acute neuritis is referred to as acute painful neuritis. The contribution from inflammation and neuropathy may vary from patient to patient and from time to time. The paresthesia complained of is usually of an annoying type, and with the advancing age of patients many other discomforts are added to it. It will be interesting to relate the paresthesia with disease activity in “cured” patients but, on the other hand, it also scares me. Any suggestion or discussion about pain might exacerbate the problems because patients are relatively unstable emotionally and tend to develop dependence because of peculiar psychosocial effects of the disease.

Mild paresthesia can be managed with suggestions and counseling, whereas disabling paresthesia needs drugs in addition. But before all that can be formulated and put into practice, treatment of leprosy has to be modified from community approach to individualized approach.

—Dr. G. N. Malaviya

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E-mail: govindmalaviya@rediffmail.com

REFERENCES

Reconstructive Surgery & Rehabilitation in Leprosy and other Neuropathies.
Richard Schwarz, and Wim Brandsma.

This 388-page multi-authored paperback focuses on aspects of surgical reconstruction and rehabilitation for impairments in leprosy and complications in diabetic feet. The editors, one a surgeon and the other a leprosy physiotherapist, have dedicated the book to the late Dr. Paul Brand who pioneered reconstructive surgery in leprosy at the Christian Medical College Hospital, Vellore, India, and who, with extraordinary foresight, concurrently introduced rehabilitation of the leprosy disabled at a “New Life Centre” within the hospital for channeling the skills of the affected individuals to safer vocations to prevent damage of their hands and feet. The Centre came to be recognized as “the birthplace of hand rehabilitation.”

The text has a wide variation in style and emphasis between chapters, which is to be expected in a book with authors of varied experience. Chapters elaborating general principles of reconstructive surgery, functional assessment, and motor and sensory assessments are well written and contain a lot of new details in the respective areas. Quick referencing would have been much easier had such useful information been placed under suitable subheadings.

A book which title ends with “other neuropathies” ideally requires a Neurologist and/or Neurophysiologist as a co-author to elaborate several neuropathies that have features akin to leprosy in order to have greater appeal in countries where leprosy is uncommon. Terms like (a)sensate and (re)occurrence rather than the accepted “insensate” and “recurrence,” respectively, are ambiguous in describing clinical states and can make for imprecise field level reporting. The chapter on Neuritis is projected well with emphasis that steroids by itself are more beneficial for early nerve impairment. I think there were grounds for stressing the need for controlled studies of fascicular nerve decompression under magnification alongside steroid therapy in early neuritis for providing a more significant sensory recovery and indeed motor improvement, since loupes and operating microscopes are used in several large centers in the developing world.

The chapters covering surgical reconstruction in the hands, feet, face, and nose are comprehensive with most segments of these texts re-emphasizing the foundation procedures, a testimony in itself to what most of these work-horse reconstructive surgeries had adequately accomplished over the past five decades. Tension adjustment techniques for tendon transfers in the hand do not include a discussion on the newer proposals to measure intraoperative sarcomere length with laser diffraction technique that also combine information on biomechanical modeling generated from normative values of the muscle architecture, tendon compliance, and joint moment in order to optimize function of transferred tendons.

The chapters on Neuropathic feet, and Management of Ulcers in the Neurologically impaired feet are chapters that stand alone, providing a welcome trend in evolving strategies for treating persistent secondary impairments of the feet. There is greater clarity on salvage procedures of Neuropathic feet and this taken together with Appendices C, D, and E makes for an excellent source of reference in managing difficult complications in leprosy and diabetic feet.

The chapter on pre- and post-operative therapy techniques already well established by physiotherapists of the past genre is revisited adequately. The chapters on Orthotics and Prosthetics, and Rehabilitation are concise and clear. The figures in the text are acceptable for the paper they have been printed on. The caveat in the line illustrations is for surgeons in training and those just starting out to realize that these are intended to conceptualize the procedure rather than provide precise technical details for obtaining the best results.
Reconstructive Surgery & Rehabilitation in Leprosy and other Neuropathies.

Reconstructive Surgery & Rehabilitation in Leprosy and other Neuropathies, edited by Schwartz and Brandsma, is a twenty-four chapter, three-hundred and eighty page text which provides a review of surgical and therapeutic modalities for the health-care providers treating leprosy. The editors are diverse in their discipline, one a surgeon and the other a physiotherapist. The editors authored some of the chapters, but solicited eighteen other professional contributors with international experience to author the majority of the chapters. Although the book is not totally comprehensive for every surgical technique or modality available, it is broad-based concerning leprosy care.

The information in each chapter is presented clearly. The material is well illustrated, including both drawings and photographs to assist the reader in understanding the surgical techniques or concept of treatment. The appendices provide sample assessment protocols as well as a section concerning casting techniques.

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—Dr. Ronnie Mathews

Orthopedic Consultant
National Hansen’s Disease Programs
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There are few typographic and syntactical errors, and wrong referencing. Other deficiencies are the absence of a chapter on operating theater techniques to aid leprosy centers; the absence of a listing and illustration of common assortments of instruments for tendon transfer surgery, bone surgery, and some special instrumentation for arthrodesis, skin grafting, and flap cover; and the absence of evaluation/grading systems to study the results of various surgeries so that young surgeons can learn from large centers, compare outcomes and improve on techniques. Contradictory remarks on internationally accepted surgical procedures are placed as editorial inserts in few areas of the text. However these deficiencies should not be considered as limiting in the importance of this book which the editors present in a 2 columns per page format at the affordable price of GBP 20.

At a time when molecular biologists are assiduously mining *Mycobacterium leprae* genome in search for better diagnostics and vaccines, and sero-epidemiologists newly research the prevention of leprosy, more ground is being covered by social and behavioral scientists to influence the course of the disease and its total control, and deformity rates are plummeting below 2 per thousand from the 20 per thousand it once was on record, the editors are to be congratulated for updating a text on reconstructive surgery for residual deformities in leprosy. This book should be in hospital libraries alongside the well known classics in leprosy reconstruction and rehabilitation so as to benefit special interest groups like young surgeons and trainees in orthopaedics, plastic and general surgery, and leprosy therapists attached to hospitals dedicated to the care of leprosy patient and the diabetic.

—Dr. George A. Anderson, M.S. Orth., D. Orth., MNAMS, M.Ch. Orth., FAMS, FACS

**Professor of Orthopaedic Surgery & Head:**

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—Dr. Ronnie Mathews

Orthopedic Consultant
National Hansen’s Disease Programs
**Notice, from Asia.**

**International Course on Rehabilitation and Prevention of Disability (RPOD) and Course in Community Based Rehabilitation (CBR)**

For many years now a much needed and very successful international course on Rehabilitation and Prevention of Disabilities (RPOD) has been conducted in Pokhara, Nepal. The international faculty are very experienced in clinical leprosy and the rehabilitation of persons affected by leprosy.

The **RPOD-Management course** will aim at teaching concepts in rehabilitation and POD, approaches to rehabilitation, rehabilitation and POD management, specifically as it relates to leprosy affected persons.

The course will include sessions about monitoring and evaluation of activities in these areas. The course will be based on the concepts and terminology used in the International Classification of Functioning, Disability and Health (ICF) published by the World Health Organization.

For a limited number of participants an opportunity will be offered for additional **in-service** training during the week(s) following the management course. The participants will be assigned on a one-to-one basis to a tutor who will guide them through a self-learning program.

Available topics include institutional rehabilitation, CBR, expanding the services of a leprosy hospital to serve people with other rehabilitation needs, agricultural rehabilitation, statistics and information systems, footwear, prosthesis and orthoses, physiotherapy and occupational therapy. These placements will be available strictly by arrangement prior to the course only.

**CBR-course.** Increasingly, rehabilitation, and prevention of disability takes place in the community with the active involvement and participation of the community. Appropriate technology is advocated, self help groups are formed, microfinance business training is started, etc. Everything is geared towards the empowerment of people with disability and the communities in which they live.

The main objective of the course is that participants will have a working knowledge of all the important aspects related to CBR. Besides plenary theoretical sessions there will be a variety of practical (skill) sessions.

For those interested in both courses in-service training if desired can also be arranged following the CBR course.

**DATES:** RPOD course 7 to 18 March, 2005 (2 weeks) [+optional week(s) if pre-arranged]

CBR course March 21 to April 1, 2005 (2 weeks) (in-service, if pre-arranged)

**TARGET GROUP:** Managers of rehabilitation and/or POD programs, senior hospital staff, senior leprosy control staff and doctors and therapists with managerial responsibilities for RPOD/ CBR activities.

**VENUE:** The Green Pastures Training Centre in Pokhara, Nepal

**COURSE FEES** (including board & lodging): $350 per week

**FURTHER INFORMATION:** Detailed information can be obtained from:

The Training Officer
GPTC
P.O. Box 28
Pokhara 33701 Nepal
tel +977 61 524562
fax +977 61 520430
E-mail: gptc@inf.org.np

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**Notice, from Africa.**

The All Africa Leprosy, TB, Rehabilitation Research and Training Center (ALERT) announces its calendar of international training courses for 2005 (see Calendar). Courses vary from 1 to 6 weeks on topics including community based rehabilitation,
basic and clinical leprosy, TB, and HIV for physicians, sention field staff, and physicians.

For further information contact the ALERT Training Division, PO Box 165, Addis Ababa, Ethiopia. Tel 251-1-211-341 FAX 251-1-211-351.
E-mail: leprosytb@elecom.net.et
Web-site: www.telecom.net.et/tdalert

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Notice, from South America
Workshop on Hansen’s Disease: The Challenge to Integrate Diagnosis and Treatment Activities into Basic Care.

Last November 2004, a workshop was held in Brasilia (DF) on the integration of leprosy services in the basic health services. Guest lecturers were Dr. S. K. Noordeen, President of ILA, and Dr. Denie Daumerie from the World Health Organization. The one-day workshop was organized by the Ministry of Health of Brazil and coordinated by Dr. Rosa Castália França Soares Ribeiro, which is leading the National Program for Leprosy Elimination in Brazil. The meeting was attended by coordinators of the state programs of elimination, consultants from PAHO and MoH, and members of municipal health services and MORHAN. Dr. Noordeen addressed the Indian experience on integration of leprosy services in the general health services, which was a valuable contribution to the discussion of this same process in Brazil. In the agenda of the workshop, it was also included the discussion of the targets and challenges for the year 2005 in what regards the elimination of leprosy in Brazil.

Successful experiences in disease control in Brazil presented at 4th Expoepi. Minister of Health Humberto Costa and Secretary of Health Surveillance Jarbas Barbosa inaugurated the 4th National Exhibition of Successful Experiences in Epidemiology and Disease Prevention and Control (Expoepi—exhibit on epidemiology experiences). The objectives of the event, the most important in its area in all of Brazil, are to bring to light and reward actions implemented in states and cities that had a positive impact on the prevention and control of diseases of importance to public health by improving the quality of epidemiological surveillance. In the competitive exhibit, 27 experiences are being presented, in nine categories, including successful experiences in the elimination of leprosy, which received one of the top prizes.

A national award to Dr. Diltor V. A. Orpomolla. During the opening ceremony
for 4th Expoepi, Minister of the Health Humberto Costa reaffirmed the federal government’s commitment to do whatever it takes to eliminate the disease. In addition, a group of scientist were honored by the Ministry of Health in the opening night and one of those was Dr. Diltor V. A. Opromolla, renowned worldwide as a symbol of the struggle against leprosy. Among many contribution of Prof. Opromolla is the introduction of rifampicin as a leading drug in the treatment of leprosy.

### Calendar of Meetings and Events

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<thead>
<tr>
<th>Day</th>
<th>Location</th>
<th>Details</th>
<th>Contact</th>
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<tr>
<td>7–18 Mar-05</td>
<td>The Green Pastures Training Centre, Pokhara, Nepal</td>
<td>International course on Rehabilitation and Prevention of Disability</td>
<td>Wim Brandsma</td>
<td><a href="mailto:gptc@inf.org.np">gptc@inf.org.np</a></td>
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<tr>
<td>21–1 Mar–Apr 05</td>
<td>The Green Pastures Training Centre, Pokhara, Nepal</td>
<td>Community Based Rehabilitation course</td>
<td>Wim Brandsma</td>
<td><a href="mailto:gptc@inf.org.np">gptc@inf.org.np</a></td>
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<td>21–8 Mar–Apr 05</td>
<td>ALERT, Addis Abbaba, Ethiopia</td>
<td>Essentials of leprosy and tuberculosis for administrative &amp; program support staff</td>
<td>ALERT Training Division</td>
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<tr>
<td>18–6 Apr–May 05</td>
<td>ALERT, Addis Abbaba, Ethiopia</td>
<td>Essentials of leprosy and tuberculosis for physicians &amp; scientists</td>
<td>ALERT Training Division</td>
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<td>28–30 Jul-05</td>
<td>Sheraton Hotel, Seattle, USA</td>
<td>US-Japan Cooperative Medical Science Program Tuberculosis/Leprosy Panel</td>
<td>Gail G. Jacobs, Program Officer, NIAID/NIH</td>
<td><a href="mailto:gg6z@nih.gov">gg6z@nih.gov</a></td>
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<tr>
<td>12–30 Sep-05</td>
<td>ALERT, Addis Abbaba, Ethiopia</td>
<td>Clinical leprosy &amp; tropical dermatology for Physicians</td>
<td>ALERT Training Division</td>
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<tr>
<td>3–28 Oct-05</td>
<td>ALERT, Addis Abbaba, Ethiopia</td>
<td>Management of combined leprosy and tuberculosis, HIV/AIDS control programmes for physicians and operational research methods in epidemiology</td>
<td>ALERT Training Division</td>
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<td>7–16 Nov–Dec 05</td>
<td>ALERT, Addis Abbaba, Ethiopia</td>
<td>Clinical leprosy and management of combined leprosy, tuberculosis, and HIV/AIDS control programmes for senior field staff</td>
<td>ALERT Training Division</td>
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<td>tba Nov-05</td>
<td>Joao Pessoa</td>
<td>10th Brazilian Congress of Hansenology</td>
<td>Francisca Estrela</td>
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CURRENT LITERATURE

This department carries selected abstracts of articles published in current medical journals dealing with leprosy and other mycobacterial diseases.

General and Historical
Chemotherapy
Clinical Sciences
Immunopathology
Leprosy
Tuberculosis
Microbiology
Leprosy
Tuberculosis
Experimental Infections and Vaccines
Epidemiology and Prevention
Rehabilitation
Other Mycobacterial Diseases
Molecular and Genetic Studies


Published reports of paleopathological analyses of skeletal collections from Central Asia are, to date, scarce. During the macroscopic examination of skeletal remains dating to the early first millennium AD from the Ustyurt Plateau, Uzbekistan, diagnostic features suggestive of leprosy were found on one individual from Devkesken 6. This adult female exhibited rhinomaxillary changes indicative of leprosy: resorption of the anterior nasal spine, rounding and widening of the nasal aperture, erosion of the alveolar margin, loss of a maxillary incisor, and inflammatory changes in the hard palate. While it is unclear whether the bones of the hands and the feet from this individual were absent as a result of collection strategy or poor preservation, lesions affecting the tibia and fibula were recorded, and the ways in which they may be related to a diagnosis of leprosy are discussed. This is the first skeletal evidence of leprosy from Central Asia and raises questions not only about the spread of the disease in the past, but also about the living conditions of what traditionally were thought of as nomadic peoples.—Authors’ Abstract


Leprosy was a well-recognized and dreaded disease in Denmark in the Middle Ages (AD 1000–1536). A large fraction of the population was affected by leprosy in the 13th century. This paper analyzes the correlation between signs of leprosy and risk of dying in the small Danish village of Tirup (AD 1150–1350). Seven different dichotomous osteological lesions indicative of leprosy are analyzed, and it is possible to score at least one of these conditions on 135 skeletons of adult or adolescent people (aged 14 or more). Scores were transformed to a statistic, lambda, indicating the likelihood that the person to whom the skeleton belonged suffered from leprosy. The analyses indicate that the prevalence of leprosy among adult people in Tirup was 26% (95% confidence interval, 17–35%). The lambda statistic indicates that people who died with signs of leprosy did not differ in the distribution of age at death from those who did not
have such signs. Skeletons showing dental enamel hypoplasia were less likely to come from skeletons with high lambda-values. The association between lambda and dental enamel hypoplasia indicates a relationship between stress in early childhood (ages 1–6 years) and subsequent development of signs of leprosy.—Authors’ Abstract


We sent a questionnaire to members of Yokohama Medical Association and Departments of University Hospital to get an overview of leprosy patients in the clinic. Yokohama Medical Association: The rate of collection was approximately 47%. Few doctors have taken medical care of Leprosy patients. Half of the doctors will take medical care, but they have little information about Leprosy. Aged doctors do not take medical care compared with young doctors. Departments of University Hospital: The rate of collection was approximately 74%. Doctors in the University Hospitals do not hesitate to take medical care of leprosy patients. Dermatologists actively take medical care and have a chance of getting information about leprosy. It is necessary to give doctors information about leprosy and its history of stigma.—Authors’ Abstract


Declining drug costs and increases in international donor interest are leading to greater availability of antiretroviral treatment programs for persons living with the human immunodeficiency virus in parts of sub-Saharan Africa. Ensuring adequate adherence to antiretroviral drug therapy is one of the principal challenges facing successful implementation in Africa, where 70% of the world’s infected persons live. Tuberculosis and leprosy are two diseases of global importance whose control programs can provide important lessons for developing antiretroviral drug adherence strategies. This paper examines various approaches used in tuberculosis and leprosy control which could help enhance adherence to antiretroviral therapy in resource-limited settings.—Authors’ Abstract


The recent excavation of a sample of 120 human skeletons from an Iron Age site in the valley of the Mun River, a tributary of the Mekong River on the Khorat Plateau in northeast Thailand, has provided the largest sample from this period in the region to date. This paper reviews three individuals from the sample with pathological changes for which the differential diagnosis includes systemic infectious disease. In two of these, both males with lesions of the hands and feet, leprosy and psoriatic arthritis are discussed as differential diagnoses, with leprosy the most probable. In the third, a female with lesions of the spine, the differential diagnosis includes tuberculosis and nonspecific osteomyelitis. Tuberculosis is the most probable diagnosis. Although the focus of this paper is a presentation of the evidence for infectious disease at Noen U-Loke, the significance of probable diagnoses of mycobacterial diseases for the history of the diseases and for prehistory in mainland Southeast Asia is also briefly discussed.—Authors’ Abstract

Chemotherapy

Tuberculosis has been increasing significantly on a world-wide absis over the past decade, but no tuberculosis-specific drugs have been discovered in 40 years. We identifies a diarylquinoline, R207910, that potently inhibits both drug-sensitive and drug-resistant Mycobacterium tuberculosis in vitro (MIC 0.06 g/ml). In mice, R207910 exceeds the bacterial activities of isoniazid and rifampin by at least 1 log. Substitution of drugs included the World health Organization’s first-line tuberculosis treatment regimen (rifampin, isoniazid, and pyrazinamide) with R207910 accelerates bactericidal activity, leading to complete culture conversion after 2 months of treatment on some combinations. A single dose of R207910 inhibits mycobacterium growth for 1 week. Plasma levels associated with efficacy in mice are well tolerated in healthy human volunteers. Mutants selected in vitro suggest the proton pump of ATP synthase to be the target for the drug.—Author’s Abstract


The purpose of this review article is to examine the various studies that have evaluated microspheres for delivery of antymycobacterial drugs. Some of the studies strictly involve the development and evaluation of microspheres for use in antymycobacterial drug delivery, whereas others actually use drug-loaded microspheres to treat mycobacterial infections in cell lines and small animals. Although there is a potential to use microspheres to treat a variety of mycobacterial infections, it appears that most of the studies so far have focused on the etiological agent of tuberculosis, Mycobacterium tuberculosis. As a result, the infectious studies presented here all entail the treatment of that mycobacterial agent. This review will address the following aspects that are important if microspheres are to be considered an acceptable therapeutic tool: 1) in vitro release characteristics, 2) delivery, release and efficacy in macrophages, 3) effectiveness in infected small animal models, 4) safe and combined use with other antymycobacterial agents, and 5) reduced toxicity. It is hoped that once all of these parameters are evaluated, a conclusion regarding the benefit of microsphere technology in the treatment of mycobacterial diseases can be reached.—Author’s Abstract


New macrolides, such as clarithromycin and azithromycin, are active agents to Mycobacterium avium complex (MAC). Both clarithromycin and azithromycin are well-known for the ability to improve the prognosis of AIDS patients with disseminated MAC infection. However, the administration of monotherapy with a macrolide is usually associated with the emergence of drug resistance after a few months of use. Therefore, the recommended treatment for MAC infection involved the use of at least two antibiotics, which includes a macrolide in combination with rifabutin, moxifloxacin and/or ethambutol. When used as prophylactic therapy in AIDS patients, azithromycin is more convenient (1200 mg, once a week) than clarithromycin (500 mg, twice a day). Ketolides are a semi-synthetic derivative of erythromycin A, which differs from erythromycin A by substitution of a 3-keto group for L-cladinose. Telithromycin has a carbamate group linked to an imidazolium and pyridium nucleus at C11–C12. In mice model, both telithromycin and ABT-733 were active in vivo against MAC.—Authors’ Abstract


The antimicrobial agents used in the treatment of mycobacterial infections have remained largely unchanged for several decades. Primary treatment of tuberculosis relies on four drugs, isoniazid, a rifamycin,
pyrazinamide, and ethambutol (or streptomycin), and generally results in >95% cure in uncomplicated tuberculosis infection. Drug resistance greatly complicates treatment of this disease. Treatment of tuberculosis caused by multiply drug-resistant strains with “second-line” drugs remains complex, and is generally tailored to the individual patient and strain. Several of the fluoroquinolones have shown promise as second line drugs for treatment of active disease and, in combination with clarithromycin or azithromycin, ethambutol, and other agents, for treatment of *Mycobacterium avium* complex infection. While large clinical trials are not possible with second line drugs, clinical treatment data are available and suggest that the quinolones have various degrees of promise in treatment of these infections. Bacterial type II DNA topoisomerases, DNA gyrase and topoisomerase IV, are the targets of quinolones, and provide the genetic basis for quinolone activity in mycobacteria. Mutations in these enzymes result in resistance, and characterization of resistant mutants allows correlation of genotype with susceptibility phenotype. Structure-activity relationship studies have provided further insight into optimal use of quinolones in mycobacterial infections. Care should be taken in treating pneumonia with fluoroquinolones if there is a degree of suspicion of tuberculosis, since quinolone monotherapy may rapidly select for quinolone resistance, thereby removing that class of antibiotic from the small range of treatment options.—Author’s Abstract


Mycobacteria are intracellular pathogens that invade and reside inside macrophages. There has been a rapid resurgence in infections caused by the genus mycobacteria. Chemotherapy of mycobacterial infections is prolonged, hepatotoxic and very often inadequate in achieving optimal drug concentrations inside the cells. Recent advances in controlled delivery systems for drugs such as liposomes have sparked a renewed interest in their potential application for the treatment of mycobacterial infections. The versatility of liposomes in incorporation of hydrophilic/hydrophobic components, non-toxic nature, biodegradability, biocompatibility and property of sustained release makes them attractive candidates for the delivery of antitubercular drugs. Liposome research in the area of mycobacterial diseases has evolved and matured through several phases; from the laboratory to the clinics. This review, thus focuses on the use of liposomes for the treatment of various types of mycobacterial diseases.—Authors’ Abstract


The present study was designed to evaluate the chemotherapeutic efficacy of poly(DL-lactide-co-glycolide) (PLG) nanoparticles (NP) encapsulating three front-line antitubercular drugs (ATDs: rifampicin, RIF; isoniazid, INH and pyrazinamide, PZA) at 2/3rd therapeutic dose. PLG nanoparticles prepared by the double emulsion and solvent evaporation technique were administered orally at 2/3rd therapeutic dose to guinea pigs. A single oral administration of the formulation resulted in sustained drug levels in the plasma for 7–12 days and in the organs for 11–14 days with a significant improvement in mean residence time as well as drug bioavailability. The administration of PLG nanoparticles every 10 days (five doses) to *Mycobacterium tuberculosis* H(37)Rv infected guinea pigs led to undetectable bacilli in the organs, as did 46 conventional doses. Therefore, nanoparticle based antitubercular chemotherapy forms a sound basis for a reduction in dosing frequency and also offers the possibility of reducing the drug dosage.—Authors’ Abstract

Mycobacteria contain an outer membrane of unusually low permeability which contributes to their intrinsic resistance to many agents. It is assumed that small and hydrophilic antibiotics cross the outer membrane via porins, whereas hydrophobic antibiotics may diffuse through the membrane directly. A mutant of *Mycobacterium smegmatis* lacking the major porin MspA was used to examine the role of the porin pathway in antibiotic sensitivity. Deletion of the mspA gene caused high-level resistance of *M. smegmatis* to 256 microg of ampicillin/ml by increasing the MIC 16-fold. The permeation of cephalaridine in the mspA mutant was reduced ninefold, and the resistance increased eightfold. This established a clear relationship between the activity and the outer membrane permeation of cephalaridine. Surprisingly, the MICs of the large and/or hydrophobic antibiotics vancomycin, erythromycin, and rifampin for the mspA mutant were increased 2- to 10-fold. This is in contrast to those for *Escherichia coli*, whose sensitivity to these agents was not affected by deletion of porin genes. Uptake of the very hydrophobic steroid chenodeoxycholate by the mspA mutant was retarded threefold, which supports the hypothesis that loss of MspA indirectly reduces the permeability by the lipid pathway. The multidrug resistance of the mspA mutant highlights the prominent role of outer membrane permeability for the sensitivity of *M. smegmatis* to antibiotics. An understanding of the pathways across the outer membrane is essential to the successful design of chemotherapeutic agents with activities against mycobacteria.—Authors’ Abstract


Modern chemotherapy has played a major role in our control of tuberculosis. Yet tuberculosis still remains a leading infectious disease worldwide, largely owing to persistence of tubercle bacillus and inadequacy of the current chemotherapy. The increasing emergence of drug-resistant tuberculosis along with the HIV pandemic threatens disease control and highlights both the need to understand how our current drugs work and the need to develop new and more effective drugs. This review provides a brief historical account of tuberculosis drugs, examines the problem of current chemotherapy, discusses the targets of current tuberculosis drugs, focuses on some promising new drug candidates, and proposes a range of novel drug targets for intervention. Finally, this review addresses the problem of conventional drug screens based on inhibition of replicating bacilli and the challenge to develop drugs that target nonreplicating persistent bacilli. A new generation of drugs that target persistent bacilli is needed for more effective treatment of tuberculosis.—Author’s Abstract

### Clinical Sciences


The case of a male patient diagnosed to have lepromatous leprosy with type 2 reaction on multibacillary multidrug therapy, with unusual, widespread involvement of genitalia in the form of plaque and nodules of leprosy over scrotum and perimeatal region of glans, necrotic lesions of erythema nodosum leprosum over scrotum, neuritis of genital branch of genitofemoral nerve bilaterally, and azoospermia, is reported.—Authors’ Abstract


The damage to the peripheral nervous system (PNS) is a marker of *Mycobacterium leprae* (*M. leprae*) infection that develops as a result of the *M. leprae* invasion to the Schwann cells. Clinical, functional (skin-deep and stimulating electromyogra-

Despite the high prevalence of leprosy in undeveloped countries, hypercalcemia secondary to leprosy is rare. One of most important mechanisms responsible for this disorder seems to be high serum concentrations of 1,25-dihydroxyvitamin D produced extrarenally by the granulomatous tissue. Serum levels of parathyroid hormone-related protein (PTHrP) have never been analyzed in this disorder. We report here a case of hypercalcemia in a patient with leprosy. Serum levels of 1,25-dihydroxyvitamin D were normal in spite of low levels of 25-dihydroxyvitamin D and acute renal failure. Suppressed serum levels of parathyroid hormone and PTHrP were also remarkable. In this case, PTHrP seems not to play an important role in the pathogenesis of hypercalcemia. Our data indicate that this disorder may be due, at least in part, to abnormal calcitriol overproduction by granulomatous tissue. Further investigations of the prevalence and pathogenesis of this type of hypercalcemia are needed.—Author’s Abstract


BACKGROUND: Leprosy is considered a chronic disabling condition. Many clinical and immunological aspects of the disease remain ill defined. AIM: The study of clinico-pathological and laboratory findings of patients with leprosy admitted to Sina Hospital, Hamadan, Iran, from 1991 to 2000. METHODS AND PATIENTS: This is a descriptive retrospective cross-sectional study. The statistical community comprised all patients diagnosed leprosy. This diagnosis was clinical and confirmed through pathology (skin-biopsy) and laboratory (peripheral smear) measures. RESULTS: In this study, the disease was more common in males than females with a mean age of 48.5 ± 16.2 years. Most of the patients were more than 40 years old. Among 12 patients in this study, six cases were urban and six cases were rural. Six cases were living in Hamadan province and two cases migrated to Hamadan province (one of them from Afghanistan and the other from Kurdestan). Clinical diagnosis was confirmed by pathology in 11 cases, but in one case the clinical diagnosis did not match the pathology. In four cases the clinical diagnosis did not match the peripheral smear. Eight cases were admitted just once. Four cases had a history of recurrence and readmission (two patients had one time recurrence and the other two patients had two recurrences). There was no difference in the clinical findings between first presentation and recur-
From the point of complication and disability, extremity disability was more common than eye disability. Increased severity of complications was found in patients with a delayed diagnosis and incomplete treatment. **CONCLUSION:** This study showed that a rapid and correct diagnosis and complete treatment was necessary for prevention of complication and disability in patients with leprosy. Skin biopsy is recommended to confirm the diagnosis in all cases of leprosy. In the absence of pathology, patients must be considered as multibacillary patients and treated as such.—**Authors’ Abstract**


A clinical descriptive study was conducted to assess the frequency and pattern of involvement of cranial nerves in leprosy and to study the relationship of cranial nerve involvement with a leprosy patch or patches on facial skin. One hundred consecutive patients of leprosy, diagnosed by clinical features and/or slit skin smear and histopathology, were studied; of these, 22 patients had cranial nerve involvement. The mean age of patients with cranial nerve involvement was 41.2 years. 16 patients (72.7%) with cranial nerve involvement were in the age-group of 20–49 years. The male-to-female ratio was 3.4:1. The mean duration of the disease in these patients was 5.73 years. The duration of the disease in the majority of patients with cranial nerve involvement was less than 5 years. Impairment of cranial nerves was seen in 12 BT patients, 6 BL patients, and 4 LL patients. No significant difference was noted between involvement of cranial nerves in PB and MB patients. Among the cranial nerves, facial nerve was the most common nerve involved (10/22), followed by olfactory (9/22), trigeminal (7/22) and auditory (3/22) nerves. Among the risk factors, it was found that facial nerve impairment was significantly associated with facial patch(es) and also type 1 lepra reaction.—**Authors’ Abstract**


Nineteen patients with pure neural leprosy were analyzed with clinical examination, electroneuromyography and histopathology of nerve biopsies. Clinical examination showed sensory loss (78.9%), paresis (78.9%), nerve enlargement (68.4%) and nerve pain (42.1%). Electroneuromyographic study revealed an axonal pattern in 18 patients (94.7%) and a demyelinating pattern in one (0.5%). Mononeuropathy multiplex was the most frequent presentation (78.9%), followed by mononeuropathy simplex (10.5%) and polyneuropathy (10.5%). The histopathological study showed the presence of inflammatory infiltrate composed of epithelioid granuloma (42.1%), mononuclear infiltrate (36.8%) or macrophages positive for bacilli (21%). Fibrosis was present in 78.9% of the biopsies. Examination of semithin sections revealed, besides inflammatory infiltrate, myelinated fiber loss (94.7%), remyelination (42%), axonal degeneration (10%) as well as regeneration (31.5%). Based on these results, the pathogenesis of leprosy neuropathy in this group of patients is briefly discussed.—**Authors’ Abstract**


We have seen 55 trophic ulcers of the heel in 2 years in our hospital, between March 2000 and February 2002. Thirty-four were chronic heel sinuses, six cases of multiple sinuses and 28 cases of single sinus of the plantar aspect of the heel. All these cases were treated by excision of the sinus, paring the prominence of the calcaneum, or excision of the cavity within the calcaneum and coverage by a rotation flap or a modification of this flap. Over the past 6 years, we have evolved a modification of
a rotation flap that requires a fusiform incision to excise the sinus, and a curved incision for the flap extending through the instep and the non-weight bearing heel. The fusiform excision, rather than the traditional triangulation, causes the flap to partly transpose rather than rotate completely. The flap is raised superficial to the plantar aponeurosis, exposing the aponeurosis from mid-sole to the heel. It is a modification of a rotation flap. The scarring over the weight-bearing sole is minimal, restricted only to the incision necessary for the excision of the heel sinus and this is its main advantage. Twenty-one of the 34 cases healed without complications. Thirteen cases had complications, of which six were treated non-operatively and seven required either a redo of the flap or another flap cover.—Authors’ Abstract


The histopathological features of skin tissue sections in patients clinically diagnosed as leprosy were correlated with the histopathological features of nerve specimens obtained from the same patients. Fifty untreated leprosy patients attending the Outpatient Department of the Department of Dermatology and Sexually Transmitted Diseases of Smt. Sucheta Kriplani and Kalawati Saran Children’s Hospitals, New Delhi, India were included in the study. On correlating the histological features of skin and nerve tissue sections, concordant findings were found in 24 out of the 50 patients (48%) but discordance between the histopathological features of skin and nerve tissue sections were found in 26 out of 50 cases (52%). Of these 26 cases, the nerve tissue histology when compared with the skin histology showed features lower down the disease spectrum in 17 (34%) cases. Seven of the 50 patients (14%) showed histological features of leprosy higher in the disease spectrum in the nerve tissue sections than in the skin biopsy sections. One patient clinically LL leprosy demonstrated histopathological features of Histoid leprosy in the skin sections and LL in the nerve sections. The remaining one patient had features of TT leprosy in the skin tissue sections while the nerve tissue histopathology showed non-specific changes. Histological features of the skin tissue sections were consistent with the clinical diagnosis in 33 out of 50 cases (66%). When the clinical groups were correlated with the histological features of the nerve tissue sections, concordance was found in 30 of the 50 cases (60%). On comparison of the histological features of skin and nerve tissue sections with the clinical diagnosis, concordance was still lower i.e., 19 out of 50 cases (38%). Thus the histological features of the skin tissue sections correlated more frequently with the clinical diagnosis than did those of the nerve sections. The importance of neural histology lies in the fact that it shows a higher BI and a lower histological grading in some cases and if not performed the lapse can result in inadequate treatment, drug resistance and even relapse.—Authors’ Abstract


Leprosy is an infectious disease of prevalence still high in endemic areas in Brazil. The neurological presentation depends on the involved nerve and is usually associated with skin lesions and the formation of multiple abscesses. We present a case of isolated tuberculoid leprosy, discuss the occurrence, the differential diagnosis and the treatment of this rare presentation and reaffirm the importance of considering leprosy in the differential diagnosis of patients with polyneuropathy or nerve enlargement with no skin lesions.—Authors’ Abstract


The present study was carried out involving 25 patients with paucibacillary leprosy who attended the outpatient department of

This paper reports the results of a study on the inter-tester reliability of the WHO disability grading system. The WHO disability grading system is the most frequently used method of grading impairment in leprosy patients. With this method, a grade of 0–2 is assigned to each of six individual body sites (both eyes, hands and feet). The maximum grade of any of these sites is used as an overall indicator of the person’s impairment status. To date, the WHO disability grading scale has not been subjected to reliability testing. The reliability of the grading system depends on the operational definitions of the grades, the way the tester interprets these definitions and the skill of the tester. It is therefore important that the definitions are unambiguous and leave as little room as possible for multiple interpretations. Three testers with varying degrees of experience did paired assessments on a total of 150 leprosy patients in the Leprosy Mission Hospital Purulia, India, using recently published operational definitions of the WHO disability grades. For every patient, they determined the maximum grade (minimum 0, maximum 2), and calculated the impairment sum-score (EHF score), adding up the six grades for eyes, hands and feet (minimum 0, maximum 12). The weighted Kappa statistic (Kw) was used as the coefficient of inter-tester reliability. A kappa of 0 represents agreement no better than chance, and 1.0 complete (chance-corrected) agreement. Kw values of ≥0.80 are considered very good and adequate for monitoring and research. Weighted Kappa analysis yielded a reliability coefficient of 0.89 (95% CI 0.84–0.94) for the maximum grade and a Kw of 0.97 (95% CI 0.96–0.98) for the EHF score. We concluded that, when using standard operational definitions, the WHO disability grading system can be used reliably in the hands of both experienced and inexperienced testers, provided adequate training has been given. Reliability should be evaluated further in a field setting, when used by primary health care workers. It is recommended that the ‘WHO disability grading’ be renamed ‘WHO impairment grading’, using the terminology as defined by the International Classification of Functioning, Disability and Health (ICF).—Authors’ Abstract


Co-infections with human immunodeficiency virus (HIV) and Mycobacterium leprae represent unique opportunities to investigate the interaction of both pathogens. We determined the immunologic, virologic, and histopathologic characteristics of 22 co-infected Brazilian patients (median age = 38 years, 81.8% males, 72.2% with paucibacillary leprosy, and 95.4% with acquired immunodeficiency syndrome). The HIV-1 sub-
types B and BF predominated in envelope and gag heteroduplex mobility analysis. Borderline tuberculoid (BT), tuberculoid, lepromatous, and indeterminate morphology with CD3+, CD8+, and CD68+ cell distributions compatible with leprosy patients not infected with HIV were observed. Histologic evidence of nerve damage was observed in BT lesions. IgM antibody to \textit{M. leprae}-specific phenolic glycolipid I was not detected. Two of six co-infected patients monitored during highly active antiretroviral therapy (HAART) developed a leprosy type 1 reaction after an increase in CD4+ cells, suggesting an immune restoration phenomenon. Clinical, immunologic, histopathologic, and virologic features among these HIV-leprosy co-infected patients indicate that each disease progressed as in single infection. However, HAART immune reconstitution may trigger potential adverse effects, such as leprosy acute inflammatory episodes.—Authors’ Abstract


Five cases of multibacillary leprosy have been diagnosed in a period of 15 years (1987–2001) at the outpatient Department of Neurology of the University Hospital Innsbruck. All patients presented with dermatological and mild to severe polyneuropathic signs and symptoms. 4/5 patients recovered fully, whereas 1 patient with an initially severe polyneuropathy showed persistent polyneuropathy as long-term sequel. The prevalence of leprosy in the catchment area of the Department of Neurology, University Hospital Innsbruck (comprising the entire province of Tyrol—650,000 inhabitants) is to be calculated as 0.5/1 million. The incidence of newly diagnosed leprosy within this province of Tyrol is 0.04/100,000/year. The aim of the presentation of these 5 patients is—beside the epidemiologic aspect—to alert all neurologists and dermatologists that this disease still exists—despite decreasing prevalence and incidence rates on a global scale; this is of particular importance since neurological long-term sequelae can only be avoided by early diagnosis.—Authors’ Abstract


Leprosy relapses are mainly due to bacillary persistence and diamino-diphenyl-sulphone (DDS) monotherapy. Case histories were examined for 33 patients with lepromatous leprosy (LL), diagnosed 7–48 years before the relapse and treated only with DDS during 4 to 38 years. Twenty-eight patients received irregular non-supervised polychemotherapy (PCT) since 1983. Five patients received only DDS, and presented relapses 13–20 years after the treatment was stopped. Relapses were diagnosed by clinical methods, including the reappearance of lesions or presence of new anesthetic areas. All cases were confirmed by bacilloscopy, and a subset of 20 cases by skin biopsy. Four patients presented indeterminate leprosy (IL) and one patient borderline tuberculoid leprosy (BT) in the biopsy. The latter 5 demonstrated presence of intraneural bacilli; the remainder were LL. Two patients relapsed even with PCT treatment. The others were cured with supervised PCT. Predisposing factors for relapses were as follows: DDS monotherapy, irregular PCT with inadequate dosage, unsupervised treatment, treatment uncompliance, and inadequate relationship between the patient and the health staff. Inspections for relapse in leprosy is recommended for in all multibacillary patients that were treated with DDS. The clinical appearance of new lesions or new anesthetic zones, the bacilloscopy and skin biopsy, used together, are effective in establishing the presence of relapses.—Authors’ Abstract


Destruction of the nasal septum and nasal bones by \textit{Mycobacterium leprae} and subsequent infection is still seen regularly in leprosy endemic areas. The social stigma associated with this deformity is significant.
Many different procedures have been developed to reconstruct the nose. Patients operated on at Anandaban Hospital and the Green Pastures Hospital and Rehabilitation Center between 1986 and 2001 were reviewed. There were 48 patients with an average age of 47 years. Five deformities were mild, 22 were moderate, 13 were severe, and eight were not graded. Bone grafting with nasolabial skin flaps was performed in 14 cases, bone grafting alone was performed in 10 cases, flaps alone were performed in seven cases, and cartilage grafting was performed in 10 cases. In three patients, a prosthesis was inserted, and in three patients a gull-wing forehead flap was performed. Overall, excellent or good cosmetic results were obtained in 83 percent of cases. Grafting with conchal cartilage was associated with the best cosmetic results and had minimal complications. Bone grafting with or without nasolabial flaps was associated with a 50 percent complication rate of infection or graft resorption. In mild to moderate deformities, cartilage grafting is recommended; for more severe deformities, bone grafting with bony fixation and skin flaps is recommended. Perioperative antibiotics must be used, and these procedures should be performed by an experienced surgeon. In very severe cases with skin deficiency, reconstruction with a forehead flap gives good results.—Authors’ Abstract


Immune reconstitution inflammatory syndrome (IRIS) occurred in 16 of 37 antiretroviral-naive patients who were treated subsequently for tuberculosis and human immunodeficiency virus (HIV) type 1 infection. IRIS was related to increases in the CD4 cell percentage and in the ratio of CD4 cells to CD8 cells after 1 month of antiretroviral therapy and to dissemination of tuberculosis. These results have implications for the diagnosis of IRIS and the understanding of its pathogenesis.—Authors’ Abstract


Chronic infection with mycobacteria is controlled by the formation of granulomas. The failure of granuloma maintenance results in reactivation of disease. Macrophages are the dominant cell type in granulomas, but CD4+ T cells are the master organizers of granuloma structure and function. Recent work points to an unrecognized role for nonspecific T cells in maintaining granuloma function in the chronic phase of infection. In addition, it has become clear that mycobacteria and host T cells collaborate in formation of granulomas. Further understanding of how nonspecific T cells contribute to granuloma formation, as well as how bacteria and T cells maintain a harmonious relationship over the life of the host, will facilitate the development of new strategies to treat mycobacterial disease.—Authors’ Abstract

Inhibition of phagolysosome biogenesis in infected macrophages is a classical pathogenesis determinant of Mycobacterium tuberculosis. In this review we primarily cover the cellular mechanisms of M. tuberculosis phagosome maturation arrest. A detailed picture is beginning to emerge, involving regulators of membrane trafficking in mammalian cells and phagosomal interactions with endosomal organelles and the trans-Golgi network. We also present a hypothesis that overlaps may exist between the mycobacterial interference with the host cell membrane trafficking processes and the targeting of the late endosomal sorting machinery by HIV during viral budding in macrophages. We propose that interference with the endosomal sorting machinery contributes to the synergism between the two significant human diseases—AIDS and tuberculosis.—Authors' Abstract


The infection of tumor necrosis factor (TNF)-deficient mice with low doses of the virulent Mycobacterium avium strain 25291 led to the appearance of necrotic granulomas at 93 days of infection, i.e., sooner than necrotic granulomas appeared in C57BL/6 animals. Additionally, TNF-deficient mice exhibited higher mycobacterial loads in the infected organs, had extremely exacerbated gamma interferon responses as evaluated in the sera of infected animals, and showed reduced survival. Thus, TNF is not required for granuloma necrosis.—Authors’ Abstract


Mycobacterium tuberculosis is an intracellular pathogen persisting within phagosomes through interference with phagolysosome biogenesis. Here we show that stimulation of autophagic pathways in macrophages causes mycobacterial phagosomes to mature into phagolysosomes. Physiological induction of autophagy or its pharmacological stimulation by rapamycin resulted in mycobacterial phagosome colocalization with the autophagy effector LC3, an elongation factor in autophagosome formation. Autophagy stimulation increased phagosomal colocalization with Beclin-1, a subunit of the phosphatidylinositol 3-kinase hVPS34, necessary for autophagy and a target for mycobacterial phagosome maturation arrest. Induction of autophagy suppressed intracellular survival of mycobacteria. IFN-gamma induced autophagy in macrophages, and so did transfection with LRG-47, an effector of IFN-gamma required for antimycobacterial action. These findings demonstrate that autophagic pathways can overcome the trafficking block imposed by M. tuberculosis. Autophagy, which is a hormonally, developmentally, and, as shown here, immunologically regulated process, represents an underappreciated innate defense mechanism for control of intracellular pathogens.—Authors’ Abstract


In this paper, we describe the development of a culture-based biochip device for rapid detection of mycobacteria in environmental samples. Individual biochips rely upon the unique paraffinophilic nature of mycobacteria to rapidly and selectively adhere to the surface of the device. We used prototype biochips to experimentally demonstrate the concept of rapid and selective detection of mycobacteria by testing pure
cultures and using epifluorescence microscopy to visualize microorganisms on the surface. As an alternative, rapid approach for identifying the biomass on the biochip surface, we used microwaves in the 10 to 26 GHz frequency range. The results of this study indicate that different microorganisms are responsible for specific shifts in resonance frequencies of a microwave cavity. By combing the semi-selective paraffin surface of the biochip with the microorganism-specific response to the microwaves, we have developed an improved analytical system with the potential to rapidly identify and enumerate mycobacteria in environmental samples in as little as 2 hr.—Authors’ Abstract


Mycobacterial diseases, including tuberculosis, leprosy, and disease due to nontuberculous mycobacteria, are the major cause of death from infectious diseases around the world. About one-third of the world population is latently infected with Mycobacterium tuberculosis. Over 8 million new cases and nearly 2 million deaths occur each year. Tuberculosis presents a significant health threat to the world. The pathogenicity of mycobacteria is related to their ability to escape killing by ingested macrophages, latent infection, and induce delayed type hypersensitivity. This has been attributed to several components of the mycobacterial cell wall, such as surface glycolipids, lipoarabinomannan, complement activation factor, heat-shock protein, and mycobacterial DNA binding protein. From the aspect of my research interests, I have focused on mycobacterial glycolipids and mycobacterial DNA binding protein in this article. Surface molecules of mycobacteria exert pleiotropic activities in both the microbe and host, and thus participate in the pathogenesis of mycobacterial diseases. The better understanding of mycobacterial pathogenicity may open the new avenue for the development of therapeutic and prophylactic interventions.—Author’s Abstract


In spite of the availability of drugs and a vaccine, tuberculosis—one of man’s medical nemeses—remains a formidable public health problem, particularly in the developing world. The persistent nature of the tubercle bacillus, with one third of the world’s population is estimated to be infected, combined with the emergence of multi drug-resistant strains and the exquisite susceptibility of HIV-positive individuals, has underscored the urgent need for in-depth study of the biology of Mycobacterium tuberculosis address the resurgence of TB. In aiming to understand the mechanisms by which mycobacteria react to their immediate environments, molecular genetic tools have been developed from naturally occurring genetic elements. These include protein expressing genes, and episomal and integrating elements, which have been derived mainly from prokaryotic but also from eukaryotic organisms. Molecular genetic tools that had been established as routine procedures in other prokaryotic genera were thus mimicked. Knowledge of the underlying mechanisms greatly expedited the harnessing of these elements for mycobacteriological research and has brought us to a point where these molecular genetic tools are now employed routinely in laboratories worldwide.—Authors’ Abstract


A major challenge in tuberculosis control is the diagnosis and treatment of latent tuberculosis infection. Until recently, there were no alternatives to the tuberculin skin test (TST) for diagnosing latent tuberculosis. However, an alternative has now emerged in the form of a new in vitro test: the interferon-gamma assay. We did a systematic review to assess the performance of interferon-gamma assays in the immunodi-
agnosis of tuberculosis. By searching databases, contacting experts and test manufacturers, we identified 75 relevant studies. The results suggest that interferon-gamma assays that use *Mycobacterium tuberculosis* specific region of difference 1 (RD1) antigens (such as early secretory antigenic target 6 and culture filtrate protein 10) may have advantages over the TST, in terms of higher specificity, better correlation with exposure to *M. tuberculosis*, and less cross-reactivity due to BCG vaccination and non-tuberculous mycobacterial infection. However, interferon-gamma assays that use RD1 antigens in isolation may maximize specificity at the cost of sensitivity. Assays that use cocktails of RD1 antigens seem to overcome this problem, and such assays have the highest accuracy. RD1-based interferon-gamma assays can potentially identify those with latent tuberculosis who are at high risk for developing active disease, but this requires confirmation. There is inadequate evidence on the value of interferon-gamma assays in the management of immunocompromised individuals, children, patients with extrapulmonary or non-tuberculous mycobacterial disease, and populations in countries where tuberculosis is endemic. Current evidence suggests that interferon-gamma assays based on cocktails of RD1 antigens have the potential to become useful diagnostic tools. Whether this potential can be realized in practice remains to be confirmed in well designed, long-term studies.—Authors’ Abstract


Understanding how pathogenic mycobacteria subvert the protective immune response is crucial to the development of strategies aimed at controlling mycobacterial infections. Prostaglandin E(2) exerts an immunosuppressive function in the context of mycobacterial infection. Because cyclooxygenase-2 (COX-2) is a rate-limiting enzyme in prostaglandin biosynthesis, there is a need to delineate the mechanisms through which pathogenic mycobacteria regulate COX-2 expression in macrophages. Our studies demonstrate that the NF-kappaB and CRE elements of the COX-2 promoter are critical to *Mycobacterium avium*-induced COX-2 gene expression. *M. avium*-triggered signaling originates at the Toll-like receptor 2 (TLR2). Ras associates with TLR2 and activates the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK), whereas tumor necrosis factor receptor-associated factor 6 (TRAF6)/transforming growth factor beta-activated kinase 1 (TAK1)-dependent signaling activates p38 MAPK. Both ERK and p38 MAPK activation converge to regulate the activation of mitogen- and stress-activated kinase 1 (MSK1). MSK1 mediates the phosphorylation of the transcription factor CREB accounting for its stimulatory effect on CRE-dependent gene expression. *M. avium*-triggered cytoplasmic NF-kappaB activation following IkappaB phosphorylation is necessary but not sufficient for COX-2 promoter-driven gene expression. MSK1 activation is also essential for *M. avium*-triggered NF-kappaB-dependent gene expression, presumably mediating nucleosomal modifications. These studies demonstrate that the nuclear kinase MSK1 is necessary in regulating the pathogen-driven expression of a gene by controlling two transcription factors. The attenuation of MSK1 may therefore have potential benefit in restricting survival of pathogenic mycobacteria in macrophages.—Authors’ Abstract


The use of volatile production patterns produced by *Mycobacterium tuberculosis* and associated bacterial infections from sputum samples were examined in vitro and in situ using an electronic nose based on a
It was possible to successfully discriminate between *M. tuberculosis* (TB) and control media, and between *M. tuberculosis* and *M. avium*, *M. scrofulaceum* and *Pseudomonas aeruginosa* cultures in the stationary phase after 5–6 hr incubation at 37°C based on 35 samples. Using neural network (NN) analysis and cross-validation it was possible to successfully identify 100% of the TB cultures from others. A second *in vitro* study with 61 samples all four groups were successfully discriminated with 14 of 15 unknowns within each of the four groups successfully identified using cross-validation and discriminant function analysis. Subsequently, lipase enzymes were added to 46 sputum samples directly obtained from patients and the head space analysed. Parallel measurements of bacterial contamination were also carried out for confirmation using agar media. NN analysis was carried out using some of the samples as a training set. Based on the NN and genetic algorithms of up to 10 generations it was possible to successfully cross-validate 9 of 10 unknown samples. PCA was able to discriminate between TB infection alone, the controls, *M. avium*, *P. aeruginosa* and a mixed infection. These findings will have significant implications for the development of rapid qualitative systems for screening of patient samples and clinical diagnosis of tuberculosis.—Authors’ Abstract


Resistance to tuberculosis (TB) is dependent on the induction of Ag-specific CD4 Th1 T cells capable of expressing IFN-gamma. Generation of these T cells is dependent upon IL-12p70, yet other cytokines have also been implicated in this process. One such cytokine, IL-27, augments differentiation of naive T cells toward an IFN-gamma-producing phenotype by up-regulating the transcription factor T-bet and promoting expression of the IL-12Rbeta2 chain allowing T cells to respond to IL-12p70. We show that the components of IL-27 are induced during TB and that the absence of IL-27 signaling results in an altered disease profile. In the absence of the IL-27R, there is reduced bacterial burden and an increased lymphocytic character to the TB granuloma. Although the number of Ag-specific CD4 IFN-gamma-producing cells is unaffected by the absence of the IL-27R, there is a significant decrease in the level of mRNA for IFN-gamma and T-bet within the lungs of infected IL-27R(−/−) mice. Ag-specific CD4 T cells in the lungs of IL-27R(−/−) also produce less IFN-gamma protein per cell. The data show that expression of IL-27 during TB is detrimental to the control of bacteria and that although it does not affect the number of cells capable of producing IFN-gamma it does reduce the ability of CD4 T cells to produce large amounts of IFN-gamma. Because IFN-gamma is detrimental to the survival of effector T cells, we hypothesize that the reduced IFN-gamma within the IL-27R(−/−) lung is responsible for the increased accumulation of lymphocytes within the mycobacterial granuloma.—Authors’ Abstract


The immune mechanisms associated with the evolution from latent to clinically active mycobacterial coinfection in human immunodeficiency virus type 1 (HIV-1)-infected humans remain poorly understood. Previous work has demonstrated that macaques infected with simian immunodeficiency virus (SIVmac) can develop persistent *Mycobacterium bovis* BCG coinfection and a fatal SIV-related tuberculosis-like disease by 4 months after BCG inoculation. In the present study, SIVmac-infected monkeys that developed clinically quiescent mycobacterial infection after BCG inoculation were followed prospectively for the reactivation of the BCG and the development of SIV-related tuberculosis-like disease. The development of clinically latent BCG coinfection in these SIVmac-infected monkeys was characterized by a change from high to undetectable levels of bacterial or-
ganisms, with or without measurable BCG mRNA expression in lymph node cells. The reactivation of clinically latent BCG coinfection and development of SIV-related tuberculosis-like disease were then observed in these SIVmac-BCG-coinfected monkeys during a 21-month period of follow-up. The reactivation of SIV-related tuberculosis-like disease in these animals coincided with a severe depletion of CD4 T cells and a loss of BCG-specific T-cell responses. Interestingly, bacterial superantigen challenge of the SIVmac-BCG-coinfected monkeys resulted in an up-regulation of clinically latent BCG coinfection, suggesting that infection with superantigen-producing microbes may increase the susceptibility of individuals to the reactivation of AIDS-related mycobacterial coinfection. Thus, reactivation of latent mycobacterial infections in HIV-1-infected individuals may result from a loss of T-cell immunity or from a superimposed further compromise of the immune system.—Authors’ Abstract


See Current Literature, Molecular and Genetic Studies, p. 86


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


We have recently shown that two subfamilies of the glycopeptidolipids (GPL) located on the surface of Mycobacterium smegmatis, along with unknown phospholipids, participate in the nonopsonic phagocytosis of mycobacteria by human macrophages (Villeneuve, et al., 2003, J. Biol. Chem., 278, 51291–300). The latter compounds were purified and identified and their molecular mechanisms of action were examined in the present study. We showed that a phospholipid mixture that derived from the methanol-insoluble fraction inhibited the phagocytosis of M. smegmatis. This inhibition was attributable to phosphatidylinositol mannosides (PIM), namely PIM2 and PIM6, since the purified phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol were inactive. This observation was confirmed using purified PIM2 and PIM6 from M. bovis BCG that decreased by half the internalization of M. smegmatis. Both glycosphospholipids also inhibited the uptake of M. tuberculosis and M. avium but had no effect on the internalization of zymosan used as a control particle of the phagocytic process.
When coated on latex beads, PIM2 and polar GPL (GPL III) favored the particle entry through complement receptor 3 (CR3). GPL III, but not PIM2, also directed particles entry through the mannose receptor (MR). Therefore, surface-exposed mycobacterial PIM and polar GPL participate to the receptor-dependent internalization of mycobacteria in human macrophages. As such, they constitute tools to dissect receptor-signalling pathway and, as inhibitors of phagocytosis, may help to design pharmacological drugs for the control of mycobacterial infections.—Authors' Abstract


We have investigated whether both primary CD8 T cell activation and CD8 T cell-mediated protection from Mycobacterium tuberculosis challenge could occur in mycobacterial-vaccinated CD4 T cell-deficient (CD4KO) mice. Different from wild-type C57BL/6 mice, s.c. vaccination with bacillus Calmette-Guerin (BCG) in CD4KO mice failed to provide protection from secondary M. tuberculosis challenge at 3 wk postvaccination. However, similar to C57BL/6 mice, CD4KO mice were well protected from M. tuberculosis at weeks 6 and 12 postvaccination. This protection was mediated by CD8 T cells. The maintenance of protective effector/memory CD8 T cells in CD4KO mice did not require the continuous presence of live BCG vaccine. As in C57BL/6 mice, similar levels of primary activation of CD8 T cells in CD4KO mice occurred in the draining lymph nodes at 3 wk after BCG vaccination, but different from C57BL/6 mice, the distribution of these cells to the spleen and lungs of CD4KO mice was delayed, which coincided with delayed acquisition of protection in CD4KO mice. Our results suggest that both the primary and secondary activation of CD8 T cells is CD4 T cell independent and that the maintenance of these CD8 T cells is also independent of CD4 T cells and no longer requires the presence of live mycobacteria. However, the lack of CD4 T cells may result in delayed distribution of activated CD8 T cells from draining lymph nodes to distant organs and consequently a delayed acquisition of immune protection. Our findings hold implications in rational design of tuberculosis vaccination strategies for humans with impaired CD4 T cell function.—Authors’ Abstract

Immunopathology (Leprosy)


Seventy-six skin biopsies that included material from 7 controls, 65 granulomatous skin lesions and 2 each of granulation tissue and chronic non-specific inflammation, were subjected to histopathological evaluation on haematoxylin and eosin and pertinent special stains. Mast cell study was done on slides stained by toluidine blue method, with special reference to their location, and morphology and cell count were done with the help of ocululomicrometer. In normal skin, mast cell density was 11.43/mm² with a range of 6–22/mm² and an S.D. of 5.94. Highest value in the whole series was seen in TVC (66/mm²), followed by lupus vulgaris (50/mm²). Mast cell counts were normal in indeterminate and TT leprosy and showed a rise over the immunological spectrum BT to LL, with values in LL being 32.86/mm² (28–40/mm²).—Authors’ Abstract


Serum levels of cytokines (IL-4, IL-5, IFN-gamma, TNF-alpha), cytokine recep-
tors (TNFR I and II) and one monokine (neopterin) were estimated in seven leprosy patients to establish disease associated markers for reversal reactions (RR). Sera were collected at diagnosis of leprosy, at the onset of reversal reaction and at different time points during and at the end of prednisone treatment of reactions. It was expected that the serum cytokine and monokine profile before and at different time points during reactions would provide guidelines for the diagnosis and monitoring of reversal reactions in leprosy. The cytokines and cytokine receptors were measured by ELISA, whereas a radioimmunoassay was used for neopterin measurement. Six of the seven patients showed increased levels of neopterin either at the onset of RR or 1 month thereafter, and levels declined on prednisone treatment to that seen at the time of diagnosis without reactions. No consistent disease associated cytokine profile was observed in these patients. Interestingly, serum TNF-alpha levels were increased in the same patients even after completion of prednisone treatment, indicating ongoing immune activity. In conclusion, this study demonstrates that despite cytokines levels in leprosy serum being inconsistent in relation to reversal reactions, serum neopterin measurement appears to be a useful biomarker in monitoring RR patients during corticosteroid therapy.—Authors’ Abstract


The alkaline single cell gel electrophoresis assay was performed on peripheral blood lymphocytes of lepromatous and tubercoid leprosy patients (untreated and those undergoing treatment) in order to ascertain whether differential damage to DNA occurs. The study group included 28 male and 2 female patients and 15 healthy males who were matched for age and socioeconomic status. The results revealed DNA damage in all patients, with a mean DNA migration length of 29.88 ± 3.39 microm and 38% of their cells damaged when compared with the respective values obtained in healthy controls (1.28 ± 0.40 microm, 5%). Multiple regression analysis for effects of confounding factors revealed antibiotic treatment in patients and alcohol consumption in controls as the only variables influencing DNA damage. In lepromatous and tubercloid patients, both untreated and those undergoing treatment, DNA damage increased significantly from that observed in control individuals, with greater increased damage in lepromatous patients. An increase in treatment time increased DNA damage linearly. Furthermore, an arbitrary classification of damaged cells (categories I–IV) was made based on observed tail lengths in leprosy patients (5.00–225.00 microm). The number of damaged cells in untreated patients was lower than in those undergoing treatment; the latter also had more cells with greater DNA migration lengths. There were no category III or IV cells in the control group. The results of the study therefore reveal that patients undergoing therapy had significantly greater DNA damage than untreated patients, indicating bacterial infection and drug therapy as the causal factors, since lepromatous-type disease is the more severe form with the patients having lower resistance to Mycobacterium leprae and requiring heavier and prolonged dosage of antibiotics. The study also corroborates that the assay offers an opportunity for correlating levels of therapy-induced DNA damage with administered dose and for modulating the dose-schedule so as to achieve lower levels of genotoxic damage.—Authors’ Abstract


Pathogenic mycobacteria survive inside macrophages and deactivate these cells, using a mechanism that is still poorly understood. Mycobacterial cell wall lipids constitute the first contact with the host cell. Although Mycobacterium leprae and M. bovis BCG share common antigens, they induce opposite inflammatory responses. Apolar M. leprae lipids have been shown to be anti-inflammatory by down-regulating macrophage activation and T-cell functions. We wonder if these lipids would influence cellular migration to BCG or to other in-
flammary agent. We investigated the effect of *M. leprae*, its lipids or delipidated bacteria on acute and chronic BCG- or carrageenan-induced pleurisy. Previous injection of intact or delipidated *M. leprae* did not alter either the BCG- or carrageenan-induced pleural inflammatory reaction. However, *M. leprae* lipids enhanced carrageenan-induced acute cellular migration without impairing BCG inflow; moreover, they reduced BCG chronic response. Together these data suggest distinct mechanisms for intracellular deactivation and pleural cell recruitment exerted by mycobacterial structures.—Authors’ Abstract


The SLAM-associated protein (SAP) regulates IFN-gamma expression in leprosy. Tuberculoid leprosy patients locally produce Th1 cytokines, while lepromatous patients produce Th2 cytokines. Signaling lymphocytic activation molecule (SLAM) and the SLAM-associated protein (SAP) participate in the differentiation process that leads to the production of specific patterns of cytokines by activated T cells. To investigate the SLAM/SAP pathway in *M. leprae* infection, we determined the expression of SAP, IFN-gamma and SLAM RNA messenger in leprosy patients. We found a direct correlation of SLAM expression with IFN-gamma expression, whereas the expression of SAP was inversely correlated with the expression of both SLAM and IFN-gamma. Therefore, our data indicate that SAP might interfere with Th1 cytokine responses while SLAM expression may contribute to Th1 responses in leprosy. This study further suggests that the SLAM/SAP pathway might be a focal point for therapeutic modulation of T cell cytokine responses in diseases characterized by dysfunctional Th2 responses.—Authors’ Abstract


Using a specific antibody (SMI 31), the state of phosphorylation of high and medium molecular weight neurofilaments (NF-H and NF-M) was studied in 22 leprous and four nonleprous human peripheral nerves by means of immunohistochemistry, sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) and Western immunoblot (WB). The results thus obtained were compared with morphological changes in the respective nerves studied through light and electron microscopy. Many of the leprous nerves showing minimal pathology revealed lack of or weak staining with SMI 31, denoting dephosphorylation. Remyelinated fibres stained intensely with SMI 31 antibody. The WB analysis of Triton X-100 insoluble cytoskeletal preparation showed absence of regular SMI 31 reactive bands corresponding to 200 and 150 kDa molecular weight (NF-H and NF-M, respectively) in 10 nerves. Three of the 10 nerves revealed presence of NF protein bands in SDS-PAGE but not in WB. Presence of additional protein band (following NF-M) was seen in four nerves. Two nerves revealed NF-H band but not NF-M band and one nerve showed trace positivity. In the remaining five nerves presence of all the three NF bands was seen. Thus, 77.3% (17/22) of human leprous nerves studied showed absence of regular SMI 31 reactive bands corresponding to 200 and 150 kDa molecular weight (NF-H and NF-M, respectively) in 10 nerves. Three of the 10 nerves revealed presence of NF protein bands in SDS-PAGE but not in WB. Presence of additional protein band (following NF-M) was seen in four nerves. Two nerves revealed NF-H band but not NF-M band and one nerve showed trace positivity. In the remaining five nerves presence of all the three NF bands was seen. Thus, 77.3% (17/22) of human leprous nerves studied showed abnormal phosphorylation of NF protein(s). The ultrastructural study showed abnormal compaction and arraying of NF at the periphery of the axons in the fibres with altered axon to myelin thickness ratio (atrophied fibres) as well as at the Schmidt-Lantermann (S-L) cleft region. Such NF changes were more pronounced in the severely atrophied axons suggesting a direct correlation. The observed well-spaced NF in the remyelinated fibers under ultrastructural study was in keeping with both intense SMI 31 staining and presence of NF triplet bands seen in WBs in four of leprous nerves that showed a large number of regenerating fibres suggesting reversal of changes with regeneration. Findings in the present study suggest that atrophy, that is, the reduction in
axoncal calibre and paranodal demyelination, seen in leprous nerves may result from dephosphorylation of NF-H and NF-M proteins.—Authors’ Abstract


While formaldehyde fixation preserves tissue morphology, it often hinders immunodetection of antigens in paraffin-embedded tissue because the antigens are masked. Antigen unmasking can be achieved with treatments such as microwave irradiation but they often lead to excessive tissue damage. Therefore, an electrochemical antigen-retrieval method (EAR) was devised in which an alternating electric current is passed through the tissue in a chamber containing an electrolyte buffer. The results obtained with this method were compared to those after microwave irradiation using archived samples of formaldehyde-fixed and paraffin-embedded lepromatous leprosy skin. The efficacy of the two unmasking procedures was assessed by the immunodetectability of several marker antigens using 24 antibodies. Fifteen antibodies that were directed against transmembrane proteins (CD), and the remaining 9 against cytokeratins 18.6 and 19, laminin, vimentin, S100a, BCG, Ulex europaeus lectin, PCNA, and P21ras. Simple and double immunohistochemistry was performed using the universal ENVISION and LSAB + AP detection systems. After unmasking with the EAR method, immunoreactivity was clearly detected with 22 of the 24 antibodies in single labeling reactions. They include the critical antigens CD3 and CD4 for identifying the T lymphocyte lineages. In contrast, only 20 of the antibodies reacted after microwave irradiation. After double immunolabeling, immunoreactivity was quantitatively similar with both methods. However, the EAR unmasking produced a stronger labeling reaction. Thus, with double labeling immunohistochemistry, EAR made it possible to use higher antibody dilutions and shorter incubation times. Heat damage was also prevented. In conclusion, EAR treatment produces better staining results than microwave irradiation treatment.—Authors’ Abstract

Immunopathology (Tuberculosis)


A new approach, short-oligonucleotide-ligation assay on DNA chip (SOLAC), is developed to detect mutations in rifampin-resistant Mycobacterium tuberculosis by short oligonucleotide ligation assay on DNA chips. The method needs only four common probes to detect 15 mutational variants of the rpoB gene within 12 hr. Fifty-five rifampin-resistant M. tuberculosis isolates were analyzed, resulting in 87.3% accuracy and 83.6% concordance relative to DNA sequencing.—Authors’ Abstract


Toll-like receptors (TLRs) such as TLR2 and TLR4 have been implicated in host response to mycobacterial infection. Here, mice deficient in the TLR adaptor molecule myeloid differentiation factor 88 (MyD88) were infected with Mycobacterium tuberculosis (MTB). While primary MyD88(−/−) macrophages and DCs are defective in TNF, IL-12, and NO production in response to mycobacterial stimulation, the upregulation of costimulatory molecules CD40 and CD86 is unaffected. Aerogenic infection of MyD88(−/−) mice with MTB is lethal
within 4 weeks with 2 log(10) higher CFU in the lung; high pulmonary levels of cytokines and chemokines; and acute, necrotic pneumonia, despite a normal T cell response with IFN-gamma production to mycobacterial antigens upon ex vivo restimulation. Vaccination with Mycobacterium bovis bacillus Calmette-Guerin conferred a substantial protection in MyD88(-/-) mice from acute MTB infection. These data demonstrate that MyD88 signaling is dispensable to raise an acquired immune response to MTB. Nonetheless, this acquired immune response is not sufficient to compensate for the profound innate immune defect and the inability of MyD88(-/-) mice to control MTB infection.—Authors’ Abstract


BALB/c mice with pulmonary tuberculosis develop a T helper cell type 1 response that peaks at 3 weeks, temporarily controlling bacterial growth. Then bacterial proliferation recommences, accompanied by increasing interleukin (IL)-4 levels and decreasing interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha, and inducible nitric oxide synthase (iNOS) levels. These changes mimic those in the human disease. In a previous study, administration of dehydroepiandrosterone (DHEA) beginning on day 60 after infection reversed these changes and protected the mice. However, DHEA is suboptimal for human use, partly because it is readily metabolized into sex steroids. 16 alpha-Bromoepiandrosterone (EpiBr; 16 alpha-bromo-5 alpha-androstan-3 beta-ol-17-one) is a synthetic adrenal steroid derivative that does not enter sex steroid pathways. In the present study, when tuberculous BALB/c mice were treated with EpiBr 3 times/week beginning on day 60, inhibition of bacterial proliferation and increased expression of TNF-alpha, IFN-gamma, and iNOS were observed, although decreased expression of IL-4 was also observed. Moreover, when given as an adjunct to conventional chemotherapy, EpiBr enhanced bacterial clearance. Trials for the use of EpiBr in the treatment of human tuberculosis are now justified.—Authors’ Abstract


Both innate and adaptive immunity play an important role in host resistance to Mycobacterium tuberculosis infection. Although several studies have suggested that the major histocompatibility complex (MHC) haplotype affects susceptibility to infection, it remains unclear whether the modulation of T-cell immunity by the MHC locus determines the host’s susceptibility to tuberculosis. To determine whether allelic differences in the MHC locus affect the T-cell immune response after M. tuberculosis infection, we infected inbred and H-2 congenic mouse strains by the respiratory route. The H-2 locus has a profound effect on the antigen-specific CD4+-T-cell response after M. tuberculosis infection. CD4+ T cells from infected mice of the H-2(b) haplotype produced more gamma interferon (IFN-gamma) after in vitro stimulation with mycobacterial antigens than mice of the H-2(k) haplotype. A higher level of IFN-gamma was also detected in bronchoalveolar lavage fluid from infected mice of the H-2(b) haplotype. A level of IFN-gamma was also detected in bronchoalveolar lavage fluid from infected mice of the H-2(b) haplotype. Furthermore, C3.SW-H2(b)/SnJ mice generate and recruit activated T cells to the lung after infection. Despite a robust immune response, C3.SW-H2(b)/SnJ mice succumbed to infection early and were similarly susceptible to infection as other C3H (H-2(k)) substrains. These results suggest that although the MHC haplotype has a profound impact on the T-cell recognition of M. tuberculosis antigens, the susceptibility of C3H mice to infection is MHC independent.—Authors’ Abstract

Optimum immunity against Mycobacterium tuberculosis requires both CD4(+) and CD8(+) T cells. In contrast with CD4(+) T cells, few antigens are known that elicit CD8(+) T cells during infection. CD8(+) T cells specific for culture filtrate protein-10 (CFP10) are found in purified protein derivative positive donors, suggesting that CFP10 primes CD8(+) T cells in vivo. Using T cells from M. tuberculosis-infected mice, we identified CFP10 epitopes recognized by CD8(+) T cells and CD4(+) T cells. CFP10-specific T cells were detected as early as week 3 after infection and at their peak accounted for up to 30% of CD8(+) T cells in the lung. IFN-gamma-producing CD8(+) and CD4(+) T cells recognizing CFP10 epitopes were preferentially recruited to the lungs of M. tuberculosis-infected mice. In vivo cytolytic activity of CD8(+) T cells specific for CFP10 and TB10.3/10.4 proteins was detected in the spleen, pulmonary lymph nodes, and lungs of infected mice. The cytolytic activity persisted long term and could be detected 260 days after infection. This paper highlights the cytolytic function of antigen-specific CD8(+) T cells elicited by M. tuberculosis infection and demonstrates that large numbers of CFP10-specific cytolytic CD8(+) T cells are recruited to the lung after M. tuberculosis infection.—Authors’ Abstract


Pulmonary macrophages provide the preferred hiding and replication site of Mycobacterium tuberculosis but display antimicrobial functions. This raises questions regarding the role of macrophages during tuberculosis. We depleted lungs of activated macrophages (activated macrophage(−) mice) and compared this with nonselective macrophage depletion (macrophage(−) mice). Although nonselective depletion of macrophages after infection improved clinical outcome, depletion of activated macrophages led to impaired resistance, reflected by enhanced mycobacterial outgrowth. The production of tumor necrosis factor-alpha and numbers of granuloma decreased after depletion of activated macrophages. Both macrophage(−) and activated macrophage(−) mice showed polarized production of interferon-gamma by splenocytes and lymph-node cells and were able to attract and activate T cells in the lung. These data demonstrate that the dual role of macrophages is associated with the activation state of macrophages and that extensive apoptosis found in patients with tuberculosis could be part of a host defense strategy, as long as these cells are not activated.—Authors’ Abstract


The effect of cortisol and/or dehydroepiandrosterone (DHEA) on the immune response to antigens obtained from Mycobacterium tuberculosis was studied in vitro by using peripheral blood mononuclear cells obtained from patients at various stages of lung tuberculosis (TB) and from healthy control people (HCo). The results obtained show for the first time that addition of cortisol within concentrations of physiological range can inhibit the mycobacterial antigen-driven proliferation of cells from HCo and TB patients and the production of interferon-gamma (IFN-gamma), indicating that endogenous levels of cortisol may contribute to the decreased lymphoid cell response to mycobacterium antigens observed in TB patients. DHEA did not affect lymphoid cell proliferation, IFN-gamma production and the cortisol-mediated inhibitory effects. Interestingly, we found that...
DHEA, but not cortisol, suppressed the in vitro transforming growth factor-beta production by lymphoid cells from TB patients with an advanced disease, which is indicative of a selective direct effect of this hormone.—Authors’ Abstract


Infection of macrophages with Mycobacterium tuberculosis or exposure to M. tuberculosis 19-kDa lipoprotein for >16 hr inhibits gamma interferon (IFN-gamma)-induced major histocompatibility complex class II (MHC-II) expression by a mechanism involving Toll-like receptors (TLRs). M. tuberculosis was found to inhibit murine macrophage MHC-II antigen (Ag) processing activity induced by IFN-gamma but not by interleukin-4 (IL-4), suggesting inhibition of IFN-gamma-induced gene regulation. We designed an approach to test the ability of M. tuberculosis-infected cells to respond to IFN-gamma. To model chronic infection with M. tuberculosis with accompanying prolonged TLR signaling, macrophages were infected with M. tuberculosis or incubated with M. tuberculosis 19-kDa lipoprotein for 24 hr prior to the addition of IFN-gamma. Microarray gene expression studies were then used to determine whether prolonged TLR signaling by M. tuberculosis broadly inhibits IFN-gamma regulation of macrophage gene expression. Of 347 IFN-gamma-induced genes, M. tuberculosis and 19-kDa lipoprotein inhibited induction of 42 and 36%, respectively. Key genes or gene products were also examined by quantitative reverse transcription-PCR and flow cytometry, confirming and extending the results obtained by microarray studies. M. tuberculosis inhibited IFN-gamma induction of genes involved in MHC-II Ag processing, Ag presentation, and recruitment of T cells. These effects were largely dependent on myeloid differentiation factor 88, implying a role for TLRs. Thus, prolonged TLR signaling by M. tuberculosis inhibits certain macrophage responses to IFN-gamma, particularly those related to MHC-II Ag presentation. This inhibition may promote M. tuberculosis evasion of T-cell responses and persistence of infection in tuberculosis.—Authors’ Abstract


The 30 kDa secreted antigen of Mycobacterium tuberculosis was purified to homogeneity by serial chromatography, and enzyme linked immunosorbent assay (ELISA) was used to evaluate its diagnostic value in patients with pulmonary tuberculosis. The immunoglobulin (Ig) antibodies G, A, and M were estimated in the two groups: patients who were smear- and culture-positive (S+C+) for pulmonary tuberculosis and normal healthy subjects (NHS). Sensitivity of 67.4%, 14.8%, and 14.3%, with the specificity of 99%, 96.7%, and 92% were obtained for the 3 isotypes respectively. Combination of the results of IgG and IgA increased the sensitivity to 71%, with 97% specificity. Polyethylene glycol precipitation of the circulating immune complexes (CIC) in sera was carried out. The CIC bound antibodies offered a sensitivity of 92.5%, 85.4%, and 68.7%, respectively for the S+C+, S–C+, and S–C-patients, while the specificity was 96.6%. Thus CIC-bound antibodies promise to be a better diagnostic tool in the detection of tuberculosis.—Authors’ Abstract


Cathepsin G (CatG) is a serine protease found in the azurophilic granules of monocytes that is known to have antimicrobial properties, but its role in Mycobacterium
tuberculosis infection is unknown. We found that *M. tuberculosis* infection of human THP-1 monocytic cells induced the down-regulation of CatG mRNA expression, as demonstrated by gene array analysis and reverse transcription-PCR. This was associated with a concomitant decrease in CatG protein and enzymatic activity. In contrast, the expression of lysosomal cathepsins B and D was up-regulated in infected cells. This effect was also observed when THP-1 cells were induced to differentiate into adherent macrophages by exposure to bacterial lipopolysaccharide (LPS). In agreement with this, CatG expression was null in adherent macrophages isolated from bronchoalveolar lavages and normal blood. We wanted to determine if the down-regulation of CatG would be relevant to *M. tuberculosis* infection. First, we found that addition of CatG to THP-1 cells prior to infection resulted in decreased bacillary viability, presumably due to extracellular killing of bacilli. However, pretreatment of cells with LPS, which decreases intracellular CatG expression, resulted in increased bacillary viability. Second, we found that CatG cationic peptides killed *M. tuberculosis* bacilli and were five- to sevenfold more bactericidal than full-length CatG. These observations suggest that *M. tuberculosis* infection of human mononuclear cells results in a “cathepsin switch” with down-regulation of CatG rendering *M. tuberculosis* bacilli more viable. Therefore, the down-regulation of CatG in macrophages is advantageous to *M. tuberculosis* bacilli and possibly is an important mechanism by which *M. tuberculosis* is able to evade the host immune defenses.—Authors’ Abstract


Iron is known to play an important role in different bacterial infections and, in particular, in their development. One example is infection with *Mycobacterium tuberculosis* where iron contributes to growth and survival of the bacteria within the host cell. The majority of studies performed on tuberculosis have focused on the direct effect of iron on bacterial growth; however, little is known about how iron modifies the mycobacterial-host interaction. In order to address this, we have investigated the effect of iron on intracellular growth of *M. tuberculosis* in J774 macrophages and the molecular mechanisms that are affected during this interaction. We observed that iron modifies intracellular growth of the mycobacteria and that their growth kinetics was modified from that observed for the extracellular situation in the presence of iron. Similarly, when iron was present during the infection, there was a reduced release of tumour necrosis factor-alpha and it was related to a higher number of bacilli inside the host cell and low expression of interleukin-1 (IL-1) and IL-6 mRNA. Hence, this work demonstrates that iron, besides promoting mycobacterial growth, also regulates the relationship between macrophage and bacteria.—Authors’ Abstract


*Mycobacterium tuberculosis*, the causative agent of tuberculosis, is a facultative intracellular pathogen that infects macrophages and other host cells. We show that sonication of *M. tuberculosis* results in the removal of material from the surface capsule-like layer of the bacteria, resulting in an enhanced propensity of the bacteria to bind to macrophages. This effect is observed with disparate murine and human macrophage populations though, interestingly, not with freshly explanted alveolar macrophages. Enhanced binding to macrophages following sonication is significantly greater within members of the *M. tuberculosis* family (pathogens) than within the *Mycobacterium avium* complex (opportunistic pathogens) or for *Mycobacterium*
smegmatis (saprophyte). Sonication does not affect the viability or the surface hydrophobicity of \textit{M. tuberculosis} but does result in changes in surface charge and in the binding of mannose-specific lectins to the bacterial surface. The increased binding of sonicated \textit{M. tuberculosis} was not mediated through complement receptor 3. These results provide evidence that the surface capsule on members of the \textit{M. tuberculosis} family may be an important virulence factor involved in the survival of \textit{M. tuberculosis} in the mammalian host. They also question the view that \textit{M. tuberculosis} is readily ingested by any macrophage it encounters and support the contention that \textit{M. tuberculosis}, like many other microbial pathogens, has an antiphagocytic capsule that limits and controls the interaction of the bacterium with macrophages.—Authors’ Abstract


Summary Diabetes mellitus is an important predisposing factor for tuberculosis. The aim of this study was to investigate the mechanism underlying this association using a murine model. Mice with streptozotocin-induced diabetes mellitus were prone to \textit{Mycobacterium tuberculosis} infection, as indicated by increased numbers of live bacteria in lung, liver and spleen. In diabetic mice, the levels of IL-12 and IFN-gamma in the lung, liver and spleen were lower than those in control animals on day 14 postinfection, while the opposite was true for IL-4 levels in the lung and liver. The expression pattern of inducible nitric oxide synthase (iNOS), in the two mice types was as for IL-12 and IFN-gamma. In addition, peritoneal exudate cells obtained from diabetic mice produced lower amounts of IL-12 and NO than those from control mice, when stimulated \textit{in vitro} with \textit{M. bovis} BCG. Spleen cells from diabetic mice infected with \textit{M. tuberculosis} produced a significantly lower amount of IFN-gamma upon restimulation with purified protein derivatives (PPD) than those from infected nondiabetic mice. Interestingly, addition of high glucose levels (33 mM) to the cultures of PPD-restimulated spleen cells reduced the synthesis of IFN-gamma only in diabetic mice, and not in nondiabetic mice. Finally, control of blood glucose levels by insulin therapy resulted in improvement of the impaired host protection and Th1-related cytokine synthesis. Our results suggest that the reduced production of Th1-related cytokines and NO account for the hampered host defense against \textit{M. tuberculosis} infection under diabetic conditions.—Authors’ Abstract
Microbiology


BACKGROUND: Analysis of variable numbers of tandem repeats (VNTR) of genetic elements called mycobacterial interspersed repetitive units (MIRUs) is a recently described, polymerase chain reaction (PCR)-based method used to genotype Mycobacterium tuberculosis. It is much faster, requires a smaller amount of DNA, and has approximately the same discriminatory power as the standard IS6110 restriction fragment-length polymorphism (RFLP) method. We report the adaptation and optimization of MIRU-VNTR genotyping on a capillary electrophoresis system. We describe its application to 3 typical clinical situations encountered in our laboratory (Institut Pasteur de Bruxelles, Laboratoire Tuberculose et Mycobacteries; Brussels, Belgium). METHODS: MIRU-VNTR genotyping was performed on heat-inactivated M. tuberculosis cultures obtained from clinical specimens on Lowenstein solid medium or in mycobacteria growth indicator liquid tubes (Becton Dickinson). After amplification of 12 genomic loci using 4 different multiplex PCRs, DNA fragments were separated by capillary electrophoresis using the ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems). Sizing of the PCR fragments and assignment of the various MIRU-VNTR alleles were done using the GeneScan and customized Genotyper software packages (PE Applied Biosystem). RESULTS: Clustering on the basis of IS6110 fingerprinting of isolates from 3 different patients attending the same hospital was confirmed by MIRU-VNTR typing. This concordance between 2 independent, highly discriminatory techniques was decisive in triggering an epidemiological inquiry that led to identification of a bronchoscopy-related tuberculosis nosocomial infection. A mixed tuberculosis infection in a patient whose infection was initially suspected as a result of the IS6110 RFLP method was clearly identified by MIRU-VNTR typing. Finally, automated MIRU-VNTR analysis permitted the identification of laboratory contamination in 6 liquid cultures of M. tuberculosis within several hours. CONCLUSION: These examples illustrate the utility of this genotyping technique for quick and accurate resolution of problems commonly encountered in clinical mycobacteriology.—Authors’ Abstract

Microbiology (Leprosy)


The relation between diaminodiphenylsulfone (called dapsone)-resistance and point mutations of the dihydropteroate synthase (DHPS) gene was analyzed using dapsone resistant Mycobacterium leprae isolates derived from Japanese leprosy patients. The mutataion was found at amino acid residues 53 or 55 of the DHPS. This finding suggests that two specific mutations in the DHPS gene involved in dapsone resistance of M. leprae.—Author’s Abstract


DNA sequences of Mycobacterium leprae in particular regions of the gyrA, rpoB, and folP genes responsible for resistance to new quinolones, rifampicin and dapsone, respectively, were analyzed. Among 88 isolates of M. leprae from leprosy patients in Japan, Haiti, Indonesia, Pakistan, and the
Philippines, eleven isolates had mutational changes in 2 genes (resistance to 2 drugs), and 2 isolates (Shinsei-1 and Zensho-4) showed mutations in 3 genes (resistance to 3 drugs). These findings are suggesting emergence of multi-drug resistant *M. leprae*.


We have searched for *Mycobacterium leprae* DNA for 35kDa protein in urine using a *M. leprae* specific PCR technique. A limited number of 16 patients (of which 11 belonged to lepromatous leprosy and five to tuberculous leprosy) and eight healthy individuals were included for the present study. The number of urine samples positive by PCR were 36.4% (4/11) in lepromatous patients and 40% (2/5) in tuberculoid patients. None of the samples from healthy individuals was positive. To our knowledge, the results indicate, for the first time, the presence of *M. leprae* DNA in urine from leprosy patients. Another important finding obtained out of the study is that amongst treated patients 66.6% (4/6) were positive whereas amongst untreated only 20% (2/10) were positive. From the present indicative data it appears that treatment improves the PCR results with urine as a sample. Thus, the approach could prove to be useful for monitoring the treatment response of individual patients and needs to be further evaluated with a large number of patients.—Authors’ Abstract


Analysis of the genome sequence of *Mycobacterium tuberculosis* H37Rv has identified 16 genes that are similar to the mammalian adenylyl and guanylyl cyclases. Rv1647 was predicted to be an active adenylyl cyclase but its position in a phylogenetically distant branch from the other enzymes characterized so far from *M. tuberculosis*, makes it an interestingly divergent nucleotide cyclase to study. In agreement with its divergence at the sequence level from other nucleotide cyclases, cloning, expression and purification of Rv1647 revealed differences in its biochemical properties from the earlier characterized Rv1625c adenylyl cyclase. Adenylyl cyclase activity of Rv1647 was activated by detergents but was resistant to high concentrations of salt. Mutations of substrate specifying residues to those present in guanylyl cyclases failed to convert the enzyme to a guanylyl cyclase, but did not alter its oligomeric status. Orthologs of Rv1647 could be found in *M. leprae*, *M. avium* and *M. smegmatis*. The ortholog from *M. leprae* (ML1399) was cloned, protein expressed, purified and shown biochemically to be an adenylyl cyclase, thus representing the first adenylyl cyclase to be described from *M. leprae*. Importantly, Western blot analysis of subcellular fractions from *M. tuberculosis* and *M. leprae* revealed that Rv1647 and ML1399 gene products were expressed in these bacteria respectively. Additionally, *M. tuberculosis* was also found to express the Rv1625c adenylyl cyclase, suggesting that multiple adenylyl cyclase proteins may be expressed simultaneously in this organism. These results suggest that Class III cyclase-like gene products are likely to have an important role to play in the physiology and perhaps the pathology of these medically important bacteria.—Authors’ Abstract


Objective. Identification of the presence and drug resistance of *Mycobacterium leprae* is key to the diagnosis and treatment of leprosy in non-endemic countries like Korea. The aim of this study was to screen the drug target DNA such as folP, rpoB, gyr, and 23S rRNA of drug resistance strain of *M. leprae*.
Patients and methods. Sequences of those genes were analyzed for the 104 bacterial index positive cases out of 171 leprosy patients in Korea using touchdown PCR, single stranded conformational polymorphism. Results. Twenty (19.2%) cases have shown the mutations in folP gene of dapsone-resistant \textit{M. leprae} in which three (2.89%) cases were mutations in two genes, folP and rpoB, of multidrug resistant strains to dapsone and rifampin, and two (1.92%) cases in folP and gyr genes of resistance to dapsone and ofloxacin, respectively. Besides double mutation for folP gene was one case (0.96%) and for rpoB gene one case, respectively. There were no mutant isolates in 23S rRNA gene against clarithromycin, Conclusions. This result should lead to a better understanding of the status of multidrug resistant leprosy in Korea and may assist in the rapid diagnosis of drug resistant \textit{M. leprae} and the choice of the appropriate treatment regimens.—Authors’ Abstract


To investigate genetic diversity in a bacterial population, we measured the copy numbers of simple sequence repeats, or microsatellites, in \textit{Mycobacterium leprae} from patients living in and around Hyderabad, India. Three microsatellite loci containing trinucleotide or dinucleotide repeats were amplified from infected tissues, and the copy numbers were established by sequence analysis. Extensive diversity was observed in a cross-sectional survey of 33 patients, but closely related profiles were found for members of a mult case family likely to share a common transmission source. Sampling of multiple tissues from single individuals demonstrated identical microsatellite profiles in the skin, nasal cavity, and bloodstream but revealed differences at one or more loci for \textit{M. leprae} present in nerves. Microsatellite mapping of \textit{M. leprae} represents a useful tool for tracking short transmission chains. Comparison of skin and nerve lesions suggests that the evolution of disease within an individual involves the expansion of multiple distinct subpopulations of \textit{M. leprae}.—Authors’ Abstract

\textbf{Microbiology (Tuberculosis)}


Human tuberculosis is a complex disease caused by bacterial populations that are located in discrete lesions (microenvironments) in a single host. Some of these microenvironments are conducive to replication, whereas others restrict bacterial growth without necessarily sterilizing the infecting microorganisms. The physical and biochemical milieu in these lesions is poorly defined. None of the existing animal models for tuberculosis (except perhaps non-human primates) reproduce the diversity of disease progression that is seen in humans. Nonetheless, transcriptomics and studies using bacterial mutants have led to testable hypotheses about metabolic functions that are essential for viability in the absence of replication. A complete picture of bacterial metabolism must balance reducing equivalents while maintaining an energized membrane and basic cellular processes.—Authors’ Abstract.


The ultrastructure of \textit{Mycobacterium tuberculosis} cells undergoing division was examined by electron microscopy. Two features of cell division were observed and are described here. First, cells are capable of undergoing a type of “snapping” post-fission movement. This movement is likely due to a multi-layered cell wall in which the inner layer participates in septum formation.
while the outer layer ruptures first on one side. A second feature related to cell division is the ability of dividing cells to form transient branching structures.—Author’s Abstract


BACKGROUND: The new challenges involved in the chemotherapy of tuberculosis make it necessary to find novel drugs, especially ones that are useful in the latent phase of the disease. METHODS: We evaluated the activity of linezolid and fluoroquinolones against logarithmic- and stationary-phase Mycobacterium tuberculosis. RESULTS: We observed that linezolid exhibits antibacterial action, although slowly, in both situations. Quinolones with an 8-methoxy group exhibit greater activity than levofloxacin in logarithmic growth phases, whereas levofloxacin exhibits greater activity in stationary-phase growth. CONCLUSION: The study of the activity of drugs against the M. tuberculosis microorganism in the latent phase is one of the most important tools available in the fight against the tuberculosis epidemic, and both linezolid and the new fluoroquinolones appear to be promising drugs.—Authors’ Abstract


Z-prenyl diphosphate synthases catalyze the sequential condensation of isopentenyl diphosphate with allylic diphosphates to synthesize polyprenyl diphosphates. In mycobacteria, these are precursors of decaprenyl phosphate, a molecule which plays a central role in the biosynthesis of essential mycobacterial cell wall components, such as the mycolyl-arabinogalactan-peptidoglycan complex and lipoarabinomannan. Recently, it was demonstrated that open reading frame Rv2361c of the Mycobacterium tuberculosis H37Rv genome encodes a unique prenyl diphosphate synthase (M. C. Schulbach, P. J. Brennan, and D. C. Crick, J. Biol. Chem. 275: 22876–22881, 2000). We have now purified the enzyme to near homogeneity by using an Escherichia coli expression system and have shown that the product of this enzyme is decaprenyl diphosphate. The enzyme catalyzes the addition of isopentenyl diphosphate to geranyl diphosphate, neryl diphosphate, omega,E,E-farnesyl diphosphate, omega,E,Z-farnesyl diphosphate, or omega,E,E,E-geranylgeranyl diphosphate, with Km values for the allylic substrates of 490, 29, 84, 290, and 40 microM, respectively. This is a first report of a bacterial Z-prenyl diphosphate synthase that preferentially utilizes an allylic diphosphate primer having the alpha-isoprene unit in the Z configuration, indicating that Rv1086 (omega,E,Z-farnesyl diphosphate synthase) and Rv2361c act sequentially in the biosynthetic pathway that leads to the formation of decaprenyl phosphate in M. tuberculosis.—[Abbreviated authors’ abstract by the Editor]


Caspase-1 is a cysteine protease composed by two 20-kDa and two 10-kDa sub-units that processes pro-IL-1beta and pro-IL-18 to their mature forms. This enzyme is present in cells as a latent zymogen that becomes active through a tightly regulated proteolytic cascade. Activation is initiated by the oligomerization of an adaptor molecule, or by the formation of a multiprotein complex named inflammasome. Negative regulation of caspase-1 activation is exerted by proteins that compete with the adaptor molecule or with the inflammasome formation. We previously reported that fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, increases caspase-1 activity in PBMC. This effect was strengthened by Mycobacterium tuberculosis, rendering an exacerbated IL-1beta, IL-18, and
IFN-gamma production. Mevalonate, the product of 3-hydroxy-3-methylglutaryl co-enzyme A reductase, is a precursor for both nonsterol isoprenoid and sterol formation. In this study, we studied the involvement of mevalonate derivatives in the regulation of caspase-1 activation. inhibition of sterol formation by SKF-104976 or haloperidol had no effect on IL-1beta release. However, the isoprenoid geranylgeraniol prevented both caspase-1 activation and the exacerbated IL production induced by fluvastatin. This isoprenoid significantly reduced the release of IL-18 and IFN-gamma by PBMC treated with mycobacteria, even in the absence of fluvastatin. In correlation with the increased caspase-1 activity, fluvastatin stimulated the proforms cleavage, enhancing the formation of active subunit p10. Geranylgeraniol not only prevented this effect, but induced proforms accumulation. Present results suggest that, once the proteolytic cascade is initiated, geranylgeraniol may exert an additional negative regulation on caspase-1 cleavage process.—Authors’ Abstract

Experimental Infections and Vaccines


Tuberculosis vaccine candidate consisting of a 72-kDa polypeptide or fusion protein based upon the Mtb32 and Mtb39 antigens of Mycobacterium tuberculosis and designated Mtbb72F was tested for its protective capacity as a potential adjunct to the Mycobacterium bovis BCG vaccine in the mouse and guinea pig models of this disease. Formulation of recombinant Mtbb72F (rMtbb72F) in an AS02A adjuvant enhanced the Th1 response to BCG in mice but did not further reduce the bacterial load in the lungs after aerosol challenge infection. In the more stringent guinea pig disease model, rMtbb72F delivered by coadministration with BCG vaccination significantly improved the survival of these animals compared to BCG alone, with some animals still alive and healthy in their appearance at >100 weeks post-aerosol challenge. A similar trend was observed with guinea pigs in which BCG vaccination was boosted by DNA vaccination, although this increase was not statistically significant due to excellent protection conferred by BCG alone. Histological examination of the lungs of test animals indicated that while BCG controls eventually died from overwhelming lung consolidation, the majority of guinea pigs receiving BCG mixed with rMtbb72F or boosted twice with Mtbb72F DNA had mostly clear lungs with minimal granulomatous lesions. Lesions were still prominent in guinea pigs receiving BCG and the Mtbb72F DNA boost, but there was considerable evidence of lesion healing and airway remodeling and reestablishment. These data support the hypothesis that the coadministration or boosting of BCG vaccination with Mtbb72F may limit the lung consolidation seen with BCG alone and may promote lesion resolution and healing. Collectively, these data suggest that enhancing BCG is a valid vaccination strategy for tuberculosis that is worthy of clinical evaluation.—Authors’ Abstract


Tuberculosis remains the leading cause of death among infectious diseases, accounting for more than two million deaths.
annually. The incidence of the disease is increasing globally, partially because of the resurgence of drug-resistant strains of *Mycobacterium tuberculosis*. Calixarenes are macrocyclic oligomers, some of which are able to modify the growth of *M. tuberculosis* in infected cells. Most experimental work has been carried out with Macrocyclon, also known as HOC 12.5EO. In this study, we demonstrate that Macrocyclon is effective in controlling *M. tuberculosis* infections, and we provide evidence that its effect is partially mediated by an l-arginine-dependent mechanism of macrophage activation that involves the activity of the inducible nitric oxide synthase. We also show that Macrocyclon is effective in athymic and major histocompatibility complex class II–/– mice and synthesized a number of structurally related calixarenes expressing significant antimycobacterial activity.—Authors’ Abstract


The fbpA and fbpB genes encoding the 85A and 85B proteins of *Mycobacterium tuberculosis* H37Rv, respectively, were disrupted, the mutants were examined for their ability to survive, and the strain lacking 85A (DeltafpA) was tested for its ability to immunize mice. The DeltafpA mutant was attenuated in mice after intravenous or aerosol infection, while replication of the DeltafpB mutant was similar to that of the wild type. Complementation of the fbpA gene in DeltafpA restored its ability to grow in the lungs of mice. The DeltafpA mutant induced a stronger expression of pulmonary mRNA messages in mice for tumor necrosis factor alpha, interleukin-1 beta (IL-1beta), gamma interferon, IL-6, IL-2, and inducible nitric oxide (NO) synthase, which led to its decline, while H37Rv persisted despite strong immune responses. H37Rv and DeltafpA both induced NO in macrophages and were equally susceptible to NO donors, although DeltafpA was more susceptible *in vitro* to peroxynitrite and its growth was enhanced by NO inhibitors in mice and macrophages. Aerosol-infected mice, which cleared a low-dose DeltafpA infection, resisted a challenge with virulent *M. tuberculosis*. Mice subcutaneously immunized with DeltafpA or *Mycobacterium bovis* BCG and challenged with *M. tuberculosis* also showed similar levels of protection, marked by a reduction in the growth of challenged *M. tuberculosis*. The DeltafpA mutant was thus attenuated, unlike DeltafpB, but was also vaccinogenic against tuberculosis. Attenuation was incomplete, however, since DeltafpA revived in normal mice after 370 days, suggesting that revival was due to immunosenescence but not compensation by the fbpB or fbpC gene. Antigen 85A thus affects susceptibility to peroxynitrite in *M. tuberculosis* and appears to be necessary for its optimal growth in mice.—Authors’ Abstract


Tuberculosis is responsible for >2 million deaths a year, and the number of new cases is rising worldwide. DNA vaccination combined with *Mycobacterium bovis* bacillus Calmette Guerin (BCG) represents a potential strategy for prevention of this disease. Here, we used a heterologous prime-boost immunization approach using a combination of DNA plasmids and BCG in order to improve the efficacy of vaccination against *Mycobacterium tuberculosis* infection in mice. As model antigens, we selected the *M. tuberculosis* Apa (for alanine-proline-rich antigen) and the immunodominant Hsp65 and Hsp70 mycobacterial antigens combined with BCG. We demonstrated that animals injected with a combination of DNA vectors expressing these antigens, when boosted with BCG, showed increased specific antimycobacterial immune responses compared to animals vaccinated with BCG alone. More importantly, the pro-
Detection achieved with this regimen was also significantly better than with BCG alone.—

Authors' Abstract


Effect of M. tuberculosis infection was studied on the expression of intercellular adhesion molecule-1 (ICAM-1) and Mac-1 markers on murine peritoneal macrophages. Intraperitoneal administration of M. tuberculosis resulted in a marked increase in the proportion of Mac-1(+) cells whereas the proportion of ICAM-1(+) cells declined sharply 4 hr post infection. Absolute numbers of Mac-1(+) and ICAM-1(+) cells however increased at all time points after the infection. Comparison of kinetics of changes observed in Mac-1(+) and ICAM-1(+) cell populations with differential leukocyte counts in peritoneal cells indicated that these alterations could be due to cellular influx, especially that of neutrophils, or up regulation of these markers on macrophages and other peritoneal cells. In adherent peritoneal macrophages infected in vitro with M. tuberculosis, proportion of Mac-1(+) and ICAM-1(+) cells increased markedly within 24 hr of infection. Mean expression of these markers on per cell basis also increased significantly. Similar results were obtained by using RAW 264.7 mouse macrophage cell line, suggesting that the enhanced expression of Mac-1 and ICAM-1 markers was a direct effect of M. tuberculosis infection and not mediated by contaminating cell types present in adherent macrophage preparations. Mac-1 and ICAM-1 expression was further studied on macrophages that had actually engulfed M. tuberculosis and compared with bystander macrophages without intracellular M. tuberculosis. For this purpose M. tuberculosis pre-stained with DilC18 fluorescent dye were used for infecting adherent peritoneal macrophages. Mac-1 and ICAM-1 expression on gated DilC18 positive and negative cell populations was analyzed. Our results indicate that the expression of Mac-1 and ICAM-1 markers was significantly enhanced on all macrophages incubated with M. tuberculosis but was more pronounced on macrophages with internalized mycobacteria. Taken together, our results suggest that the expression of Mac-1 and ICAM-1 markers is significantly up regulated as a result of exposure and infection with M. tuberculosis. Since these markers play important role in the uptake of mycobacteria as well as in the process of antigen presentation by macrophages, their upregulation may be beneficial for generation of a protective immune response to M. tuberculosis.—Authors’ Abstract


Infection of C57BL/6 mice with Mycobacterium avium leads to the activation of both CD4+ and CD8+ gamma interferon (IFN-gamma)-producing T cells, although the CD8+ cells play no role in protection against infection. Using transfer of different lines of transgenic T cells with T-cell receptors (TCRs) which recognize irrelevant antigens, we show here that transferred CD8+ T cells from two of the three lines were activated to the same degree as the host cells, suggesting that the majority of the IFN-gamma-producing CD8+ T cells of the host represented bystander activation. The third line, specific for the male HY antigen, showed no activation. Activation required the participation of the CD28 co-receptor on T cells and was unaffected by the removal of CD44(hi) (memory phenotype) T cells. The transferred CD8+ T cells proliferated in vivo, although this was not essential for IFN-gamma production. Taken together, these data are highly reminiscent of homeostatic proliferation of TCR transgenic T cells upon transfer to lymphopenic hosts, and suggest low-affinity stimulation through the TCR, possibly by self peptides. The findings are discussed in relation to homeostatic proliferation and their significance in the possible induction of autoimmune disease.—Authors’ Abstract

Izzo, A. A., Izzo, L. S., Kasimos, J., and Majka, S. A matrix metalloproteinase

OBJECTIVE: The host response to pulmonary *Mycobacterium tuberculosis* (Mtb) infection results in granuloma formation in an effort to limit infection, but the host immune cells also provide an environment in which Mtb persists. Granuloma formation requires immune cell infiltration and concurrent extensive remodeling of pulmonary tissue which we hypothesize to be the result of increased matrix metalloproteinases (MMP) activity. DESIGN: C57BL/6 mice infected with virulent Mtb (H37Rv) via intratracheal inoculation were treated with a synthetic inhibitor of MMP activity (BB-94). Mice were assessed for colony forming units, granuloma morphology, leukocyte recruitment and cytokine levels over 90 days of infection. RESULTS: BB-94 treated mice had significantly decreased numbers of pulmonary and blood-borne Mtb early during disease, increased collagen deposition within early granulomas and significantly decreased pulmonary leukocyte recruitment when compared to vehicle-treated, Mtb-infected mice. Cytokine expression did not differ significantly between groups. CONCLUSION: Events of early granuloma formation can be modified by inhibiting MMP activity, by decreasing leukocyte recruitment, a major source of MMPs during infection, enhancing the establishment of granulomas and decreasing blood-borne dissemination of Mtb.—Authors’ Abstract


Live mycobacteria have been reported to signal through both Toll-like receptor 2 (TLR2) and TLR4 *in vitro*. Here, we investigated the role of TLR2 in the long-term control of the infection by the attenuated Mycobacterium, *Mycobacterium bovis* BCG, *in vivo*. We sought to determine whether the reported initial defect of bacterial control (K. A. Heldwein, *et al*., *J. Leukoc. Biol*. 74:277–286, 2003) resolved in the chronic phase of BCG infection. Here we show that TLR2-deficient mice survived a 6-month infection period with *M. bovis* BCG and were able to control bacterial growth. Granuloma formation, T-cell and macrophage recruitment, and activation were normal. Furthermore, the TLR2 core-
ceptor, TLR6, is also not required since TLR6-deficient mice were able to control chronic BCG infection. Finally, TLR2-TLR4-deficient mice infected with BCG survived the 8-month observation period. Interestingly, the adaptive response of TLR2- and/or TLR4-deficient mice seemed essentially normal on day 14 or 56 after infection, since T cells responded normally to soluble BCG antigens. In conclusion, our data demonstrate that TLR2, TLR4, or TLR6 are redundant for the control of *M. bovis* BCG mycobacterial infection.—Authors’ Abstract


A fusion protein of antigen 85B (Ag85B) and ESAT-6 administered in cationic lipid vesicles conferred a highly significant level of protection against *Mycobacterium tuberculosis* in the guinea pig aerosol model of infection. The protection was manifested as delayed clinical illness and prolonged survival. Neither Ag85B nor ESAT-6 (independently or as a cocktail) induced significant protection in this model.—Authors’ Abstract


Tuberculosis (TB) remains a threat for public health, killing around 3 million people a year. Despite the fact that most cases can be cured with antibiotics, the treatment is long and patients relapse if chemotherapy is not continued for at least 6 months. Thus, a better characterization of the working principles of the immune system in TB and identification of new immunotherapeutic products for the development of shorter regimens of treatment are essential to achieve an effective management of this disease. In the present work, we demonstrate that immunotherapy with a plasmid DNA encoding the *Mycobacterium leprae* 65 kDa heat-shock protein (hsp65) in order to boost the efficiency of the immune system, is a valuable adjunct to antibacterial chemotherapy to shorten the duration of treatment, improve the treatment of latent TB infection and be effective against multidrug-resistant bacilli (MDR-TB). We also showed that the use of DNA-hsp65 alone or in combination with other drugs influence the pathway of the immune response or other types of inflammatory responses and should augment our ability to alter the course of immune response/inflammation as needed, evidencing an important target for immunization or drug intervention.—Authors’ Abstract


Mycobacteria are intracellular pathogens that invade and reside inside the macrophages. Recent advances in controlled delivery systems for vaccines such as liposomes have sparked a renewed interest in their potential application for the prevention of mycobacterial infections. The versatility of liposomes in the incorporation of hydrophilic/hydrophobic components, their non-toxic nature, biodegradability, biocompatibility, adjuvanticity, induction of cellular immunity, property of sustained release and prompt uptake by macrophages, makes them attractive candidates for the delivery of antigens. This review focuses on liposome research in the area of mycobacterial diseases and highlights how the various mycobacterial components may be exploited as powerful antigens with liposomes as adjuvants.—Authors’ Abstract

Xue, T., Stavropoulos, E., Yang, M., Ragno, S., Vordermeier, M., Chambers, M., Hewinson, G., Lowrie, D.

We have previously demonstrated that vaccination of mice with plasmid DNA vectors expressing immunodominant mycobacterial genes induced cellular immune responses and significant protection against challenge with *Mycobacterium tuberculosis*. We demonstrate here, using in vitro-synthesized RNA, that vaccination with DNA or RNA constructs expressing the *M. tuberculosis* MPT83 antigen are capable of inducing specific humoral and T-cell immune responses and confer modest but significant protection against *M. tuberculosis* challenge in mice. This is the first report of protective immunity conferred against intracellular bacteria by an RNA vaccine. This novel approach avoids some of the drawbacks of DNA vaccines and illustrates the potential for developing new antimycobacterial immunization strategies.—Authors’ Abstract

Epidemiology and Prevention


DNA samples from blood and nasal swabs of 125 healthy household contacts was submitted to amplification by polymerase chain reaction (PCR) using a *Mycobacterium leprae*-specific sequence as a target for the detection of subclinical infection with *M. leprae*. All samples were submitted to hybridization analysis in order to exclude any false positive or negative results. Two positive samples were confirmed from blood out of 119 (1.7%) and two positive samples from nasal secretion out of 120 (1.7%). The analysis of the families with positive individuals showed that 2.5% (N = 3) of the contacts were relatives of multibacillary patients while 0.8% of the cases (N = 1) had a paucibacillary as an index case. All positive contacts were followed up and after one year none of them presented clinical signs of the disease. In spite of the PCR sensitivity to detect the presence of the *M. leprae* in a subclinical stage, this molecular approach did not seem to be a valuable tool to screen household contacts, since we determined a spurious association of the PCR positivity and further development of leprosy.—Authors’ Abstract


LEC s were carried out from 1998 to 2000 in eight counties of west China. The number of cases detected during the year of LECs was much higher than that detected by routine methods before the year of the LEC. However, the annual number of cases detected during the year after the LEC showed different patterns. One pattern is that the number of new cases detected in the year after the LEC declined to the level similar to that before the year of the LEC. The second pattern is that the number of new cases detected in the year after the LEC declined steeply to less than that detected before the year of the LEC. Following peak case-detection during the year of the LEC, a gradual decrease in the number of new cases was observed in the subsequent years. The repeat LEC brought a weakly rebounding peak case-detection during the year following the first LEC carried out 3 years earlier. The operational, epidemiological and technical factors influencing the trends of case-detection during the LECs are discussed.—Authors’ Abstract

Estimating the economic value to societies of health research is a complex but essential step in establishing and justifying appropriate levels of investment in research. The practical difficulties encountered include: identifying and valuing the relevant research inputs (when many pieces of research may contribute to a clinical advance); accurately ascribing the impact of the research; and appropriately valuing the attributed economic impact. In this review, relevant studies identified from the literature were grouped into four categories on the basis of the methods used to value the benefits of research. The first category consists of studies that value direct cost savings that could arise from research leading either to new, less-costly treatments or to developments such as vaccines that reduce the number of patients needing treatment. The second category comprises studies that consider the value to the economy of a healthy workforce. According to this “human capital” approach, indirect cost savings arise when better health leads to the avoidance of lost production. The third category includes studies that examine gains to the economy in terms of product development, consequent employment and sale. The studies placed in the fourth category measure the intrinsic value to society of the health gain, by placing a monetary value on a life. The review did not identify any consistency of methodology, but the fourth approach has most promise as a measure of social value. Many of the studies reviewed come from industrialized nations and proposal is made by the present reviewers for an international initiative, covering developed and developing countries, to understand further methodology analysis and testing.—Tropical Diseases Bulletin


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

The current classification of non-pigmented and late-pigmenting rapidly growing mycobacteria (RGM) capable of producing disease in humans and animals consists primarily of three groups, the Mycobacterium fortuitum group, the Mycobacterium chelonae-abscessus group and the Mycobacterium smegmatis group. Since 1995, eight emerging species have been tentatively assigned to these groups on the basis of their phenotypic characters and 16S rRNA gene sequence, resulting in confusing taxonomy. In order to assess further taxonomic relationships among RGM, complete sequences of the 16S rRNA gene (1483–1489 bp), rpoB (3486–3495 bp) and recA (1041–1056 bp) and partial sequences of hsp65 (420 bp) and sodA (441 bp) were determined in 19 species of RGM. Phylogenetic trees based upon each gene sequence, those based on the combined dataset of the five gene sequences and one based on the combined dataset of the rpoB and recA gene sequences were then compared using the neighbor-joining, maximum-parsimony and maximum-likelihood methods after using the incongruence length difference test. Combined datasets of the five gene sequences comprising nearly 7000 bp and of the rpoB+recA gene sequences comprising nearly 4600 bp distinguished six phylogenetic groups, the M. chelonae-abscessus group, the Mycobacterium mucogenicum group, the M. fortuitum group, the Mycobacterium mageritense group, the Mycobacterium wolinskyi group and the M. smegmatis group, respectively comprising four, three, eight, one, one and two species. The two protein-encoding genes rpoB and recA improved meaningfully the bootstrap values at the nodes of the different groups. The species M. mucogenicum, M. mageritense and M. wolinskyi formed new groups separated from the M. chelonae-abscessus, M. fortuitum and M. smegmatis groups, respectively. The M. mucogenicum group was well delineated, in contrast to the M. mageritense and M. wolinskyi groups. For phylogenetic organizations derived from the hsp65 and sodA gene sequences, the bootstrap values at the nodes of a few clusters were <70%. In contrast, phylogenetic organizations obtained from the 16S rRNA, rpoB and recA genes were globally similar to that inferred from combined datasets, indicating that the rpoB and recA genes appeared to be useful tools in addition to the 16S rRNA gene for the investigation of evolutionary relationships among RGM species. Moreover, rpoB gene sequence analysis yielded bootstrap values higher than those observed with recA and 16S rRNA genes. Also, molecular signatures in the rpoB and 16S rRNA genes of the M. mucogenicum group showed that it was a sister group of the M. chelonae-abscessus group. In this group, M. mucogenicum ATCC 49650(T) was clearly distinguished from M. mucogenicum ATCC 49649 with regard to analysis of the five gene sequences. This was in agreement with phenotypic and biochemical characteristics and suggested that these strains are representatives of two closely related, albeit distinct species.—Authors’ Abstract


A nonphotochromogenic, rapidly growing Mycobacterium strain was isolated in pure culture from the sputum and the bronchoalveolar fluid of a patient with hemoptoic pneumonia by using axenic media and an amoebal coculture system. Both isolates grew in less than 7 days at 24 to 37°C with an optimal growth temperature of 30°C.
The isolates exhibited biochemical and antimicrobial susceptibility profiles overlapping those of *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium immunogenum*, indicating that they belonged to *M. chelonae-M. abscessus* group. They differed from *M. abscessus* in beta-galactosidase, beta-N-acetyl-beta-glucosaminidase, and beta-glucuronidase activities and by the lack of nitrate reduction and indole production activities, as well as in their *in vitro* susceptibilities to minocycline and doxycycline. These isolates and *M. abscessus* differed from *M. chelonae* and *M. immunogenum* by exhibiting gelatinase and tryptophane desaminase activities. Their 16S rRNA genes had complete sequence identity with that of *M. abscessus* and >99.6% similarity with those of *M. chelonae* and *M. immunogenum*. Further molecular investigations showed that partial hsp65 and sodA gene sequences differed from that of *M. abscessus* by five and three positions over 441 bp, respectively. Partial rpoB and recA gene sequence analyses showed 96 and 98% similarities with *M. abscessus*, respectively. Similarly, 16S–23S rRNA internal transcribed spacer sequence of the isolates differed from that of *M. abscessus* by a A→G substitution at position 60 and a C insertion at position 102. Phenotypic and genotypic features of these two isolates indicated that they were representative of a new mycobacterial species within the *M. chelonae-M. abscessus* group. Phylogenetic analysis suggested that these isolates were perhaps recently derived from *M. abscessus*. We propose the name of “*Mycobacterium massiliense*” for this new species. The type strain has been deposited in the Collection Institut Pasteur as CIP 108297(T) and in Culture Collection of the University of Goteborg, Goteborg, Sweden, as CCUG 48898(T).—Authors’ Abstract


*Mycobacterium kansasii* is one of the most pathogenic and frequent nontuberculous mycobacteria isolated from humans. Patients with adverse drug reactions, resistant isolates, or suboptimal response require alternative treatment regimens. One hundred forty-eight consecutive clinical isolates of *M. kansasii* were tested for antimicrobial susceptibilities by the BACTEC 460 system (NCCLS) with two different inoculation protocols, one conventional and one alternative. In the alternative protocol, the inoculum 12B vial was incubated until the growth index was between 250 and 500. Four conventional antimycobacterial drugs (isoniazid, rifampin, streptomycin, and ethambutol) were studied with standard critical concentrations. The *in vitro* activities of linezolid, telithromycin, clarithromycin, levofloxacin, and moxifloxacin were determined by measuring radiometric MICs. All isolates tested were identified as *M. kansasii* genotype I and were resistant to isoniazid at a concentration of 0.4 μg/ml. One hundred twenty isolates (81.1%) were inhibited by 1 μg of isoniazid per ml. A high level of resistance to isoniazid (>10 μg/ml) was observed in six isolates (4.1%). Only five strains (3.4%) were resistant to rifampin (>1 μg/ml). All isolates studied were susceptible to streptomycin and ethambutol. The MICs at which 90% of the isolates were inhibited (in micrograms per milliliter) were as follows: linezolid, 1 (range, ≤0.25 to 2); telithromycin, >16 (range, 4 to >16); clarithromycin, 0.5 (range, ≤0.03 to 1); levofloxacin, 0.12 (range, 0.12 to 0.25); and moxifloxacin, 0.06 (range, ≤0.06 to 0.12). The susceptibility testing results with both inoculation protocols showed perfect correlation. In conclusion, all *M. kansasii* isolates showed decreased susceptibility to isoniazid, but resistance to rifampin was infrequent. Quinolones, especially moxifloxacin, were the most active antimycobacterial agents tested, followed by clarithromycin. Linezolid also showed good activity against these microorganisms, but telithromycin’s *in vitro* activity was poor.—Authors’ Abstract

**Chacon, O., Bermudez, L. E., and Bartletta, R. G.** Johne’s disease, inflammatory bowel disease, and *Mycobacterium*

Johne’s disease is a chronic diarrhea affecting all ruminants. *Mycobacterium avium* subsp. paratuberculosis (MAP), a slowly growing mycobacteria, is the etiologic agent. There is also a concern that MAP might be a causative agent of some cases of inflammatory bowel disease in humans, especially Crohn’s disease. Food products including pasteurized bovine milk have been suggested as potential sources of human infection. This review addresses microbial factors that may contribute to its pathogenicity. In addition, the experimental evidence defining MAP as the cause of Johne’s disease and the issues and controversies surrounding its potential pathogenic role in humans are discussed.—Authors’ Abstract


*Mycobacterium fortuitum* is a rapidly growing mycobacterium found in soil and water throughout the world. It can cause diseases in immunocompetent patients, usually resulting in localized skin and soft tissue infections. Cervical lymphadenitis caused by *M. fortuitum* is rare. We report a 46-year-old woman in whom skin lesions of cutaneous polyarteritis nodosa, leucocytoclastic vasculitis and Sweet’s syndrome had successively developed before the diagnosis of cervical lymphadenitis caused by *M. fortuitum* was made. The skin lesions responded to colchicine and systemic corticosteroids but recurred intermittently. After establishment of the diagnosis, she received treatment with clarithromycin and ciprofloxacin. The cervical lymph nodes decreased in size 6 months later and no more new skin lesions were found.—Authors’ Abstract


Hypermorphic mutations of the nuclear factor kappaB essential modulator gene cause ectodermal dysplasia and immunodeficiency. Affected patients have increased susceptibility to mycobacterial disease including cutaneous manifestations. We describe clinical and histopathologic characteristics of 5 patients with nuclear factor kappaB essential modulator gene mutations and mycobacterial infections, two of whom had mycobacterial cutaneous infections.—Authors’ Abstract


During the 5-year period, 1997–2001, 1700 patients with a clinical diagnosis of *Mycobacterium ulcerans* disease [Buruli ulcer (BU)] were treated at the Centre Saniataire et Nutritionnel Gbemoten, Zagnanado, Benin. The patients lived in the four regions of southern Benin: Atlantique, Mono, Oueme and Zou, with the largest number coming from the Zou Region where the center is located. The median age of BU patients was 15 years (q1 = 7, q3 = 30). Lower limbs are involved 3.2 times more frequently than upper limbs in older patients and younger patients have the highest prevalence of multiple lesions. The latter are frequently associated with bone lesions. Specific detection rates for age and gender showed a distribution with maximum peaks in the 10–14 years group and among adults between 75 and 79 years. Over 59 years, males are more at risk of developing *M. ulcerans* disease than females. Children under 15 years represent the largest part of the BU disease burden and of the general population. The highest detection rates (per 100,000 population) were in the 75–79-year-old patients. The most likely explana-
tion of this was reactivation of disease from a latent infection of *M. ulcerans*. Educational programs should target especially these two groups of population at risk.—Authors’ Abstract


This report describes detailed taxonomic and phylogenetic analysis of 15 non-tuberculous mycobacteria (NTMs) isolated from human pathological specimens in a Caribbean setting (12 slow-growers and three rapid-growers) that were not identified by cultural and biochemical tests and drug-susceptibility results. These isolates were further studied using PCR restriction fragment length polymorphism analysis (PRA) of a 441bp hsp65 fragment, as well as the sequencing of 16S rDNA and hsp65 DNA, and HPLC of the mycolic acids. Our results showed that taxonomic position of well-defined NTMs was resolved by PRA and sequencing of hsp65, nonetheless, it was not suitable to investigate rarely observed or new strains that required 16S rDNA sequencing and HPLC for a definite response. Unrooted neighbor-joining phylogenetic trees were drawn based upon the 16S rDNA and hsp65 sequences of the 15 NTMs compared with those from described species (73 for 16S rDNA and 45 for hsp65). For most of the NTMs not showing an exactly matching sequence with either hsp65 or 16S rDNA in the GenBank, the phylogenetic tree was able to provide with useful indications about their relatedness to known species. In such a case, a concordant HPLC pattern with the sequence data and the place of the strain within the tree could lead to a potential identification. Unrooted neighbor-joining phylogenetic trees were drawn based upon the 16S rDNA and hsp65 sequences of the 15 NTMs compared with those from described species (73 for 16S rDNA and 45 for hsp65). For most of the NTMs not showing an exactly matching sequence with either hsp65 or 16S rDNA in the GenBank, the phylogenetic tree was able to provide with useful indications about their relatedness to known species. In such a case, a concordant HPLC pattern with the sequence data and the place of the strain within the tree could lead to a potential identification. 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avium infection is described. Further history revealed disseminated mycobacterial infections in the patient’s father and uncle, starting at 9 years old and 1 year old, respectively. Autosomal dominant interferon-gamma receptor mutation was subsequently identified. Clinical variability among the affected members of the same family is consistent with previous reports suggesting substantial variability in the clinical course of this disorder.—Authors’ Abstract


Mycobacterial infections are increasing in incidence worldwide, partly as a result of the increase in immunocompromised individuals. They cause a large number of cutaneous infections with a broad array of manifestations. Because of their diverse manifestations and sometimes fastidious nature, infections with mycobacteria are often misdiagnosed, leading to delay in and sometimes failure of therapy. In addition, many mycobacteria display both in vitro and in vivo drug resistance to antimicrobial agents. Early recognition of affected patients, initiation of appropriate antimicrobial therapy based on current guidelines, and tailoring of therapy after susceptibility testing is available are therefore essential to the successful treatment of mycobacterial infections.—Authors’ Abstract


The membership list of genus mycobacterium is ever expanding and it has grown to 95 in year 2003. While leprosy and tuberculosis are specific diseases caused by mycobacteria, other members are usually saprophytes but can be opportunistic and at times deadly pathogens. These other mycobacteria are referred to as atypical mycobacteria, non-tuberculous mycobacteria (NTM) or mycobacteria other than tubercle bacilli (MOTT). These organisms can produce localized disease in the lungs, lymph glands, skin, wounds or bone. Occasionally they may produce disseminated disease. Of the more than 90 known species of NTM, about one third have been associated with disease in humans. The species causing human disease are: Mycobacterium avium, M. intracellulare, M. kansasii, M. paratuberculosis, M. scrofulaceum, M. simiae, M. habana, M. interjectum, M. xenopi, M. heckeshornense, M. szulgai, M. fortuitum, M. immunogenum, M. chelonae, M. marinum, M. genavense, M. haemophilum, M. celatum, M. conspicuum, M. malmoense, M. ulcerans, M. smegmatis, M. wolinskyi, M. goodii, M. thermoresistible, M. neoaurum, M. vaccae, M. palustre, M. elephantis, M. bohemicam and M. septicum. Isolation of these mycobacteria from representative specimens and their rapid identification is very important as the treatment strategy for tuberculosis and other mycobacterioses is different. Several biochemical, chemical (lipid) and molecular techniques have been developed for rapid identification of these species. Along with suggestive clinical features, poor response to antitubercular treatment and repeated isolation of the organisms from the clinical specimens these techniques can help in establishing correct diagnosis. Further, many drugs like rifampicin, rifabutin, ethambutol, clofazimine, amikacin, new generation quinolones and macrolides effective against mycobacterial infections are available that can be used in appropriate combinations and dosage to treat these infections.—Author’s Abstract


BACKGROUND: Mycobacterial infections of a pacemaker insertion site are very rare clinical events. Such infections are caused primarily by staphylococci and streptococci and, less frequently, Gram-negative organisms. CASE REPORT: We describe a case of pacemaker infection caused by Mycobacterium abscessus which is, to our knowledge, only the second such case described in the literature. The patient responded well to removal of the pacemaker wire and treatment with six months of clarithromycin. CONCLUSIONS: My-
crobacteria have been very infrequently re-
ported as causes of pacemaker infections.
To our knowledge, only one case of such
infection caused by *M. abscessus* has been
described in the literature. Herein we pre-
sent the second case of a patient with pace-
maker infection caused by *Mycobacterium
abscessus*. This case underscores the impor-
tance of considering atypical mycobacteria
in pacemaker infections, particularly if the
Gram stain or the standard cultures are neg-
ative. Removal of the contaminated foreign
body seems to be an integral part of suc-
cessful management.—Authors’ Abstract

**Lee, S. A., Raad, I. I., Adachi, J. A., and
Han, X. Y.** Catheter-related bloodstream
infection caused by *Mycobacterium bruma-
5429–5431.

*Mycobacterium brumae* is a rapidly grow-
ing environmental mycobacterial species
identified in 1993; so far, no infections by this
organism have been reported. Here we pre-
sent a catheter-related *M. brumae* blood-
stream infection in a 54-year-old woman with
breast cancer. The patient presented with high
fever (39.7 °C), and >1000 colonies of *M.
brumae* grew from a quantitative culture of
blood drawn through the catheter. A paired
peripheral blood culture was negative, how-
ever, suggesting circulational control of the
infection. The patient was treated empirically
with meropenem and vancomycin, and the
fever resolved within 24 hr. The catheter was
removed a week later, and from the tip *M.
brumae* was isolated a second time, suggest-
ing catheter colonization. The organism was
identified by colonial morphology, sequence
analysis of the 16S rRNA gene, and bio-
chemical tests.—Authors’ Abstract

**McShane, H., Pathan, A. A., Sander, C.
R., Keating, S. M., Gilbert, S. C., Hu-
ygen, K., Fletcher, H. A., and Hill, A. V.
S.** Recombinant modified vaccinia virus
Ankara expressing antigen 85A boosts
BCG-primed and naturally acquired ant-
timycobacterial immunity in humans. Nature

No Abstract Available.

**Manfredi, R., Nanetti, A., Valentini, R.,
Ferri, M., Morelli, S., and Calza, L.**
Epidemiological, clinical and therapeutic
features of AIDS-related *Mycobacterium
kansasii* infection during the HIV pan-
demic: an 11-year follow-up study. HIV

OBJECTIVES: Optimal diagnosis and
timely treatment of atypical mycobacterio-
sis, and especially *Mycobacterium kansasii*
disease, remain a serious challenge for clin-
cians engaged in the management of the
immuno-compromised host. METHODS
AND RESULTS: From more than 2700
hospitalizations (over 1800 patients) attrib-
utable to HIV-associated disorders over an
11-year period, 12 patients were found to
have a confirmed *M. kansasii* infection.
This reflects the recent reduction in the fre-
cuity of this HIV-related complication,
which virtually disappeared after the intro-
duction of potent antiretroviral combina-
tions in 1996. In the early 1990s, the lack of
effective antiretroviral regimens made fre-
quent the association with AIDS, a mean
CD4 lymphocyte count of nearly 20
cells/microL, and an extremely variable
chest X-ray features. The recent detection
of a further case was attributable to late
recognition of very advanced HIV disease,
complicated by multiple opportunistic dis-
orders. CONCLUSIONS: *Mycobacterium
kansasii* respiratory or disseminated infec-
tion continues to occur, and poses diagno-
sic problems in terms of late or missed iden-
tification as a result of slow culture and
frequently concurrent opportunistic disease.
Serious therapeutic difficulties also arise
from the unpredictable *in vitro* antimicro-
bial susceptibility profile of these organ-
isms, and from the need to start an effective
combination therapy that does not interfere
with other medications as soon as possi-
ble.—Authors’ Abstract

**Martin-Casabona, N., Bahrmand, A. R.,
Bennedsen, J., Thomsen, V. O., Cur-
cio, M., Fauville-Dufaux, M., Feld-
man, K., Havelkova, M., Katila, M. L.,
Koksalan, K., Pereira, M. F., Ro-
drighes, F., Pfyffer, G. E., Portaels, F.,
Urgell, J. R., Rusch-Gerdes, S., Tor-
toli, E., Vincent, V., Watt, B.; and the

OBJECTIVE: To collect data on non-tuberculous mycobacteria (NTM) isolated from clinical laboratories in different countries to establish: 1) whether the isolation of NTM was increasing, 2) which species were increasing, and 3) whether there was any pattern of geographical distribution.

DESIGN: In 1996, the Working Group of the Bacteriology and Immunology Section of the International Union Against Tuberculosis and Lung Disease contacted 50 laboratories in different countries for the necessary information.

RESULTS: The number of patients reported with NTM was 36099 from 14 countries. Mycobacterium avium complex, M. gordonae, M. xenopi, M. kansasii and M. fortuitum were the five species most frequently isolated. There was a significant upward trend for M. avium complex and M. xenopi. Pigmented mycobacteria predominated in Belgium, the Czech Republic and the Mediterranean coast of Spain. Non-chromogenic mycobacteria were found to be predominant in the area of the Atlantic coast of Brazil and in Turkey, the United Kingdom, Finland and Denmark.

CONCLUSIONS: There was an increase in the number of NTM isolated from clinical samples of patients. Isolation of the most frequent species is constantly changing in most of the geographical areas, and newer species are emerging due to better diagnostic techniques to detect and identify NTM.—Authors’ Abstract


Objective Tenosynovitis of the hand due to atypical mycobacteria is an uncommon condition. We present a case of tenosynovitis of the hand due to Mycobacterium chelonae in a patient without a recognized penetrating injury, who was treated successfully with clarithromycin and antituberculous medications and without debridement. We reviewed the available literature to summarize the experience with this infectious entity. Methods Case report and review of the literature (MEDLINE 1976–2003). Only cases that were sufficiently detailed were included. Results Twelve cases of upper extremity infection due to M. chelonae have been reported: hand tenosynovitis in most and arthritis in a few. These infections resulted from percutaneous inoculation or hematogenous seeding. The clinical course was indolent initially but insidiously destructive. Previously, treatment always included surgical excision of the infected tissues and antibiotic therapy. This is the first case of M. chelonae musculoskeletal infection that resolved with only antimicrobial therapy. Conclusions Musculoskeletal infections by nontuberculous mycobacteria are clinically indistinguishable from those of tuberculosis and diagnosis is usually delayed. Prompt diagnosis of atypical mycobacteria with appropriate antimicrobial treatment may avoid the need for surgical debridement. Relevance We recommend a trial of antibiotics for M. chelonae before surgical debridement.—Authors’ Abstract

Molecular & Genetic Studies


See Current Literature, Epidemiology and Prevention, p. 76


Twenty-seven polymorphisms from 12 genes have been investigated for association with tuberculosis (TB) in up to 514
cases and 913 controls from Karonga district, northern Malawi. Homozygosity for the complement receptor 1 (CR1) Q1022H polymorphism was associated with susceptibility to TB in this population (odds ratio [OR] = 3.12, 95% Confidence interval [CI] = 1.13–8.60, p = 0.028). This association was not observed among human immunodeficiency virus (HIV)-positive TB cases, suggesting either chance association or that HIV status may influence genetic associations with TB susceptibility. Heterozygosity for a newly studied CAAA insertion/deletion polymorphism in the 3′-untranslated region of solute carrier family 11, member 1 (SLC11A1, formerly NRAMP1) was associated with protection against TB in both HIV-positive (OR = 0.70, 95% CI = 0.49–0.99, p = 0.046) and HIV-negative (OR = 0.65, 95% CI = 0.46–0.92, p = 0.014) TB cases, suggesting that the SLC11A1 protein may have a role in innate TB immune responses that influence susceptibility even in immunocompromised individuals. However, associations of other variants of SLC11A1 with TB reported from other populations were not replicated in Malawi. Furthermore, associations with vitamin D receptor, interferon-gamma, and mannose-binding lectin observed elsewhere were not observed in this Karonga study. Genetic susceptibility to TB in Africans appears polygenic. The relevant genes and variants may vary significantly between populations, and may be affected by HIV infection status.—Authors’ Abstract


See Current Literature, Immunopathology, p. 54


Trends in increased tuberculosis infection and a fatality rate of approximately 23% have necessitated the search for alternative biomarkers using newly developed postgenomic approaches. Here we provide a systematic analysis of Mycobacterium tuberculosis (Mt) by directly profiling its gene products. This analysis combines high-throughput proteomics and computational approaches to elucidate the globally expressed components of the three subcellular compartments (the cell wall, membrane, and cytosol) of Mt. We report the identifications of 1044 proteins and their corresponding localizations in these compartments. Genome-based computational and metabolic pathways analyses were performed and integrated with proteomics data to reconstruct response networks. From the reconstructed response networks for fatty acid degradation and lipid biosynthesis pathways in Mt, we identified proteins whose involvements in these pathways were not previously suspected. Furthermore, the subcellular localizations of these expressed proteins provide interesting insights into the compartmentalization of these pathways, which appear to traverse from cell wall to cytoplasm. Results of this large-scale subcellular proteome profile of Mt have confirmed and validated the computational network hypothesis that functionally related proteins work together in larger organizational structures.—Authors’ Abstract


The etiology of Crohn’s disease in humans is largely unknown. Clinical signs of Crohn’s disease partly resemble the clinical picture of Johne’s disease in ruminants caused by Mycobacterium avium subsp. paratuberculosis. Because of the high prevalence of these bacteria in (products of) ru-
minants and their remarkable thermostability, concern has been raised about the possible role of these bacteria in the pathogenesis of Crohn’s disease. In an attempt to develop a molecular typing method to facilitate meaningful comparative DNA fingerprinting of \textit{M. avium} subsp. paratuberculosis isolates from the human and animal reservoirs, multilocus variable-number tandem-repeat analysis (MLVA) was explored and compared to IS900 restriction fragment length polymorphism (RFLP) typing. MLVA typing subdivided the most predominant RFLP type, R01, into six subtypes and thus provides a promising molecular subtyping approach to study the diversity of \textit{M. avium} subsp. paratuberculosis. — Authors’ Abstract


The ability to develop adequate immunity to intracellular bacterial pathogens is unequally distributed among human beings. In the case of tuberculosis, for example, infection with \textit{Mycobacterium tuberculosis} results in disease in 5–10% of exposed individuals, whereas the remainder control infection effectively. Similar interindividual differences in disease susceptibility are characteristic features of leprosy, typhoid fever, leishmaniasis, and other chronic infectious diseases, including viral infections. The outcome of infection is influenced by many factors, such as nutritional status, co-infections, exposure to environmental microbes, and previous vaccinations. It is clear, however, that genetic host factors also play an important part in controlling disease susceptibility to intracellular pathogens. Recently, patients with severe infections due to otherwise poorly pathogenic mycobacteria (non-tuberculous mycobacteria or \textit{Mycobacterium bovis} BCG) or \textit{Salmonella} spp. have been identified. Many of these patients were unable to produce or respond to interferon gamma, due to deleterious mutations in genes that encode major proteins in the type 1 cytokine (interleukin 12/interleukin 23/interferon gamma) axis (interleukin 12p40/interleukin 23p40, IL12 receptor beta1/IL23 receptor beta1, interferon gamma receptors 1 and 2, or signal transducer and activator of transcription 1). This axis is a major immunoregulatory system that bridges innate and adaptive immunity. Unusual mycobacterial infections were also reported in several patients with genetic defects in inhibitor of NFkappaB kinase gamma, a key regulatory molecule in the nuclear factor kappaB pathway. New findings discussed in this review provide further and sometimes surprising insights into the role of type 1 cytokines, and into the unexpected heterogeneity seen in these syndromes. — Authors’ Abstract


\textit{See Current Literature, Microbiology (Leprosy), p. 68}


\textit{See Current Literature, Microbiology (Leprosy), p. 69}
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