CURRENT LITERATURE

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

General and Historical

Baral, J. P., Bhattarai, S., Thapa, M. R., Ghimire, K. and Burathoki. Effectiveness of training of basic health workers in leprosy control programme. Indian J. Lepr. **70** Suppl. (1998) 23S–31S.

This study was undertaken in two adjacent districts (Rautahat and Parsa) in Nepal to measure the impact of training of basic health workers on Leprosy Control Programme. Knowledge, attitude and leprosy service delivery by them were studied before and after training. There was an improvement in all three components after training. However, improvement was also seen in the control group as well. Possible reasons for this are discussed. Improper selection of the area and an inadequate methodology were the identified drawbacks of the study.—Authors' Abstract

Brahmachari, N. S. R., Anantharaman, D. S., Rao, B. R., Gupte, M. D., Rao, S. K. and Mahalingam, V. N. Underutilization of the available services by the needy disabled leprosy patients in government leprosy control unit, Puttoor, Chittoor District, Andhra Pradesh, South India. Indian J. Lepr. **70** Suppl. (1998) 47S–61S.

This study was undertaken to find out the deformity profile, utilization of disability care services, factors associated with underutilization and the impact of educating leprosy patients with visible disabilities in self-care practices in the area covered by the LCU Chittoor. The disability prevalence rate in the area was 15 per 10,000 population. Training of the staff and teaching leprosy patients in self-care practices has shown a remarkable improvement in skin texture and ulcer situation of disabled leprosy patients.—Authors' Abstract

Deenabandhu, D. A., Narendra Babu, C. R. and Vijayakumaran, P. Study of the problem of hidden cases of leprosy among "inaccessible" section of urban populations in Madras City. Indian J. Lepr. **70** Suppl. (1998) 79S–82S.

A questionnaire survey was conducted among 1300 general practitioners (GPs) of Madras city to assess the magnitude of the leprosy problem among the upper middle class and upper class populations who are getting treatment from these GPs. A total of 2944 leprosy patients were being treated by 2000 GPs. One third of these patients belonged to the upper middle/upper class section of the population living in the city of Madras.—Authors' Abstract

Hatano, K. Leprosy. Jpn. J. Trop. Med. Hyg. **27** (1999) 87–91.

This paper covers the following aspects: characteristics of leprosy; leprosy in the world; leprosy in Bangladesh; and the merits and demerits of the WHO program on leprosy control.—Trop Dis. Bull. **96** (1999) 813

Joshi and Revankar, C. R. Improving compliance of leprosy patients with disabilities for disability care and prevention of disability services. Indian J. Lepr. **70** Suppl. (1998) 39S–45S.

This study was undertaken to identify the extent of problems such as inadequate disability prevention services and poor compliance of the disabled leprosy patients and also to develop solutions in Ulhasnagar block of Thane district, Maharashtra, India. A total of 233 patients out of 269 noncolony patients were evaluated before and after the interventions. The interventions

consisted of staff training, educating the patient and his family and provision of supplies and aids. Almost all patients complied with the given advice and were using the aids provided. Improvement was noted in physical, social and disability status. About 25% indicated that they could now undertake activities of daily life and 43% stated that they could attend to their vocation. Substantial improvement in service provider's knowledge and skill was observed. Long-term follow up is necessary to determine the sustainability of results.—Authors' Abstract

Masih, V. K., Gandhi, B. S. and Reddy, B. N. Can general practitioners carry out drug delivery and periodic examination of leprosy patients in Raipur City? Indian J. Lepr. **70** Suppl. (1998) 5S–9S.

An intervention study to improve the MDT coverage of leprosy patients by involving general practitioners (GPs) was carried out in Raipur city. Most of the GPs (84%) were willing to undertake drug delivery and periodic examination of the leprosy patients. After involving GPs, the proportion of the patients registered for treatment increased from 33% to 89.2%. The cohort regularity was only 57% which was lower than that found in the Upgraded Urban Leprosy Centre. Drug compliance as found by the pill count was 91%. Most of the patients (87%) were happy with the services provided by the GPs.—Authors' Abstract

Mall, R. P., Trivedi, D. S., Anand Kumar, S. and Girdhar, B. K. Study of low productive value of rapid survey as a means of leprosy case detection in Kanpur Urban Project. Indian J. Lepr. **70** Suppl. (1998) 33S–38S.

A study was undertaken in Kanpur city to identify the reasons for low yield of rapid survey in leprosy case detection and to intervene to overcome the shortcomings. By a random cluster sampling method, 200,000 persons were selected for the study. Rapid survey was undertaken in half the area and in another half a similar survey was undertaken after additional inputs. The additional inputs were staff training, IEC activities, changing of the survey timings and addition of a female worker to the survey teams. The proportion of the population enumerated showed a significant rise (from 58.35% to 72.21%) in the test area with additional inputs. The number and the type of cases detected did not show any difference. Significantly, the addition of female workers to the team did not improve the proportion of the female population examined or of female cases detected.—Authors' Abstract

Merlin, V. E., Miceli, I., Litturi, M., Gomez, A. and Chuit, R. Factors that make difficult the implementation of an integrated leprosy control programme in Health Zone II, Santa Fe Province, Argentina. Indian J. Lepr. **70** Suppl. (1998) 83S–95S.

With the help of a pre-tested, structure questionnaire and participatory observation, effects of several variables that have a bearing on the process of integration were studied in Zone II of the Santa Fe Province of Argentina. Patient's knowledge and the presence of an NGO were identified as factors facilitating integration. The presence of a vertical program staff and insufficient commitment toward integration were identified as factors hindering integration.—Authors' Abstract

Pangi, C., Shwe, T., Win, D. L. L., Saw, W. W., Gyi, K. K., Yee, M., Myint, Y. Y. and Htay, T. T. A comparative study of intervention methods (full, partial and non-integration) on late case detection and treatment irregularity in Yangon, Myanmar. Indian J. Lepr. **70** Suppl. (1998) 97S–105S.

The high percentage (20%) of new cases with grade 2 disabilities and a low treatment regularity of 47% indicated problems in case detection and case holding in urban Yangon. The fact that Urban Health Centers (UHCs) were not involved in the leprosy control program might have had an adverse influence. To compare the effectiveness of two methods of integration (full and partial) of urban leprosy services in terms of early case detection and regularity of treatment, this study was conducted in an urban area. Two townships with similar leprosy prevalence, staff infrastructure, socio-economic

status, transport, communication and working capacity of the Township Medical Officers (TMOs) were chosen for this intervention study: UHC-A(Thingangyun) for full integration and UHC-B(Tamwe) for partial integration and the remaining 14 townships as nonintegrated areas served by the Central Special Skin Clinic (CSSC).

This study has shown that it was possible to fully integrate the Leprosy Control Programme (LCP) into the Urban Health Centres [Basic Health Services (BHS)] in urban Yangon. Case detection could be improved by active case finding, such as contact examination and school examination conducted by the personnel of UHCs. Treatment regularity was found to be directly proportional to prompt defaulter retrieval action and the motivational level of the TMO and peripheral BHS workers. There were more complaints from patients (8.1%) treated at UHC-A when compared to CSSC (6.7%). Among defaulters there were more adults than children, more males than females and more PB than MB patients.—Authors' Abstract

Pennini, S. N., Pedrosa, L. and Rebello, P. F. B. Early diagnosis and treatment of leprosy in intradomiciliary contacts in a high prevalence area: Amazon region. Indian J. Lepr. **70** Suppl. (1998) 73S–77S.

The importance of dermato-neurological examination of intradomiciliary contacts is well known as an important secondary preventive measure in leprosy control, due to the fact that it allows early diagnosis and treatment. This is an intervention trial in an area of high leprosy prevalence (Manaus/Brazil) where the proportion of contacts examined is low. The aim of the study is to assess whether a simple educational session conducted among patents increases contacts examination and leads to early case detection. The intervention group had examined more contacts ($p < 0.05$) but, paradoxically, presented fewer new cases than the control group. The authors discuss the probable causes for this unexpected outcome, the advantages of the intervention and other related issues.—Authors' Abstract

Raja Rao, B. and Krishnamurthy, P. A comparative study of the cost and effectiveness of a modified system of MDT drug delivery system in a high endemic district (Nalagonda) of South India. Indian J. Lepr. **70** Suppl. (1998) 63S–71S.

Fall in the case load (from 17,000 to 4500), has changed the disease profile and the introduction of fixed duration Therapy (FDT) has made management of leprosy cases rather easy in Nalgonda, a backward district in Andhra Pradesh. The system of drug delivery which was conceived for managing a large case load, however, remains unchanged, thereby resulting not only in considerable waste of resources but also in hampering other activities such as case detection and patient care. This study was undertaken to develop and assess a modified system of drug delivery in terms of the cost and effectiveness, its overall effect on other activities in the program and its acceptability by the field staff. Four Leprosy Control Units (LCUs) were selected and were randomly assigned either to study (Gudibanda, Suryapet) or control (Nalgonda, Bhuvanagiri) group. In the study group the modified drug delivery system replaced the existing system. The modified system consisted of the paramedical worker being made responsible for patients at all the DDPs in his subcenter. The clinics were managed alternately by medical officers and nonmedical supervisors every month. In the control group each clinic was managed by medical officers every month and it covered two subcenters with each drug delivery point being assisted by a paramedical worker.

The study revealed that the modified system resulted in a saving of 130 man-days a month, a 30% saving in use of vehicle, a 30% saving in POL, and improvement in case detection. There was no change in the clinic attendance and drug consumption compliance in the units where modified system was introduced.—Authors' Abstract

Ramesh, V. K., Mani, R. and Parkash, I. A study of low clinic attendance of leprosy patients: reasons and solutions. Indian J. Lepr. **70** Suppl. (1998) 17S–21S.

In this study, the effect of health education on a sample of 325 absentee leprosy patients was assessed in a leprosy-endemic area. About 46% of the absentees from the study group returned to the clinic following health education (HE). HE was more effective among those who defaulted in the later part of their treatment sessions. The monthly attendance rate increased from 70%–74% to 72%–91% following HE. Among the absentees, 58% were absent due to personal reasons and 8% due to health service-related reasons. Personal reasons was the commonest cause for absenting at second pulse. At third pulse it was due to socio-economic reasons. At fourth pulse it was due to service-related reasons. At fifth pulse the commonest reason was disease-related. Health education had proved to be a definite solution to solve the absentee problem.—Authors' Abstract

Selvaraj, G., Prabakar, N., Muliyil, J. and Martin, G. Incidence of disabilities among multibacillary cases after initiation of multidrug therapy and factors associated with the risk of developing disabilities. Indian J. Lepr. **70** Suppl. (1998) 11S–16S.

Out of the 1724 new cases registered between 1985 to 1992, 1169 could be contacted. The overall incidence of disabilities was 6.8%. Age above 45 years, bacteriopositivity and thickening of three or more trunk nerves were associated with a higher risk of disabilities. Staff training, patient education and steroid availability in the field were the suggested methods of reducing the occurrence of disabilities in leprosy patients.—Authors' Abstract

Chemotherapy

Dhople, A. M. *In vitro* activity of epiroprim, a dihydrofolate reductase inhibitor, singly and in combination with brodimoprim and dapsone, against *Mycobacterium leprae.* Int. J. Antimicrob. Agents **12** (1999) 319–323.

The antimicrobial effects of a new dihydrofolate reductase inhibitor, epiroprim, alone and in combination with dapsone and brodimoprim against *Mycobacterium leprae* were evaluated *in vitro* in a cell-free culture system. Two biochemical parameters were used to measure metabolic activity (and growth) of the organism. The minimal inhibitory activity of epiroprim against *M. leprae* was 10 mg/l and the action was bactericidal. When combined with dapsone, epiroprim exhibited a strong synergism; on the other hand, combination of epiroprim and brodimoprim provided only additive effects. The results suggest that epiroprim can be a component in multidrug therapy regimen in leprosy.—Author's Abstract

Gallo, M. E. N., Alvim, M. F. S., Nery, J. A. and Albuquerque, E. C. A. [Comparative study of two multidrug therapy regimens (fixed duration) in multibacillary leprosy—follow up at 50.32 ± 19.62 and 39.70 ± 19.47 months.] Hansenol. Int. **22** (1997) 5–14. (in Portuguese)

This study compares the bacilloscopic and clinical evolution of 140 multibacillary (MB) leprosy patients from Brazil, who were divided into 2 groups and submitted to 2 treatment regimens with fixed dosage multidrug therapy (MDT). In group I, 70 cases received rifampin (RMP) 600 mg and dapsone (DDS) 100 mg daily for 3 consecutive months, followed by DDS 1000 mg/day self-administered for 21 months. Group II patients received RMP 600 mg and clofazimine (CLO) 300 mg once a month under supervision, plus self-administered doses of DDS 100 mg and CLO 50 mg daily, with 24 supervised doses duration. No statistically significant differences were found ($p > 0.05$) on neuromotor and bacilloscopic evolution between the 2 groups, neither during treatment or on follow up after discharge. Group I showed a significantly greater number of reactional cases ($p < 0.05$), during treatment and after discharge, which was attributed to CLO presence in Group II therapeutic regimen.

Group I: total follow up was 2.110 patients/year with mean 50.32 ± 16.62 to the WHO-recommended MDT regimen for MB patients, total follow up was 1.897 patients/year, mean 39.70 ± 19.47 months, and no relapse was diagnosed. In 1 of the relapse cases, a skin biopsy was inoculated into foot pads of mice (Shepard's technique) to verify bacillary viability and drug sensitivity to RFM and DDS. Results suggested that in this case, the relapse was due to the resurgence of persistent bacilli sensitive to the drugs used. After the initiation of the standard WHO/MDT both cases had a satisfactory evolution.—Trop. Dis. Bull. **96** (1999) 831–832

Gillespie, S. H. and Billington, O. Activity of moxifloxacin against mycobacteria. J. Antimicrob. Chemother. **44** (1999) 393–395.

Moxifloxacin is an 8-methoxyquinolone compound with activity against a wide range of bacteria. We tested its activity in comparison with four other quinolones and isoniazid against clinical isolates of mycobacteria. It proved to be the most active of the quinolones tested against *Mycobacterium tuberculosis* (MIC90 0.25 mg/L), *M. avium-intracellulare* (MIC90 1.0 mg/L), *M. kansasii* (MIC90 0.06 mg/L) and *M. fortuitum* (MIC90 1 mg/L). These data indicate that moxifloxacin merits further study as an antimycobacterial agent.—Authors' Abstract

Gupta, U. D., Katoch, K., Singh, H. B., Natrajan, M., Sharma, V. D. and Katoch, V. M. Detection of viable organisms in leprosy patients treated with multidrug therapy. Acta Leprol. **11** (1999) 89–92.

Cutaneous biopsies were collected from multibacillary leprosy patients who attended the outpatient department of Jalma Institute for treatment at different time intervals, i.e., 6 months, 12 months, 18 months, 24 months, 30 months, 36 months and 42 months after starting multidrug therapy (MDT) when they were still skin-smear positive. Biopsies were processed for inoculation into mouse foot pad (MFP) and estimation of bacillary ATP levels by bioluminescent assay (ATP assay) by earlier established procedures. Viable bacilli were detectable after 1 year (25% cases by MFP and 31% cases by ATP assay), 2 years (8% cases by MFP and 12% cases by ATP assay) and 3 years (4% cases by both MFP and ATP assays). Overall, the percentage of the persisters was 10% by MFP and 13% by ATP assay. It would be important to carry out surveillance studies in a larger number of BL/LL cases to know the trends and also the resultant relapses.—Authors' Summary

Lu, M., Poloyac, S. M., McNamara, P. J. and Blouin, R. A. The effect of pregnenolone 15 alpha-carbonitrile on the pharmacokinetics and metabolism of dapsone in rats. J. Pharm. Pharmacol. **51** (1999) 803–810.

The purpose of this study was to evaluate the effect of pregnenolone 16 alpha-carbonitrile (PCN) on the interconversion pharmacokinetics and metabolism of dapsone. To determine microsomal CYP3A activity and protein, eight rats (4 PCN, 4 corn oil) received a 1 mg kg^{-1} intravenous bolus dose of dapsone, followed by blood and urine sampling. The formation clearance of dapsone hydroxylamine (CLf DDS-NOH) was calculated from the obtained samples. Interconversion pharmacokinetics estimates were obtained after 10 rats (5 PCN, 5 control) received 1 mg kg^{-1} dapsone or 1.17 mg kg^{-1} monoacetyldapsone, with a 24-hr wash-out.

Results from the interconversion analysis demonstrated that PCN significantly increased systemic clearance (CLs) of dapsone, but not its interconversion. The *in-vivo/in-vitro* correlation study demonstrated that PCN significantly increased CLs of dapsone (8.55 to 16.39 mL min^{-1}; $p < 0.01$) and CLf DDS-NOH (0.13 to 0.18 mL min^{-1}; $p < 0.01$. PCN treatment produced a 69% increase in CYP3A protein, and increased 6 beta- and 2 beta-hydroxytestosterone formation rates. Significant correlations were found between CLf DDS-NOH and either 6 beta- ($r^2 = 0.925$), 2 beta-hydroxytestosterone ($r^2 = 0.92$), or CYP3A1/2 protein ($r^2 = 0.60$).

We conclude that PCN treatment produces significant increases in CLs (dapsone) and CLf (DDS-NOH) in rats. These

changes were not due to changes in the reversible metabolism of dapsone. These results suggest that the formation clearance of dapsone hydroxylamine reflects alterations in CYP3A activity, despite the fact that it accounted for a small part of the systemic clearance of dapsone.—Authors' Abstract

Prabhakaran, K., Harris, E. B. and Randhawa, B. Postantibiotic effect of ampicillin/sulbactam against mycobacteria. Microbios **99** (1999) 113–122.

The postantibiotic effect (PAE) is an important pharmacodynamic property of antibiotics. Most drugs continue to exert a suppressive effect on the growth of bacteria, both *in vitro* and *in vivo,* even after the drug concentrations have fallen below detectable levels. Only limited information is available on the PAE of slow-growing organisms like mycobacteria. The PAE of ampicillin/sulbactam (Unasyn®) was investigated against six species of mycobacteria, *Mycobacterium avium, M. africanum, M. bovis* BCG, *M. simiae, M. scrofulaceum* and *M. tuberculosis* H37Ra, by spectrophotometry. The cell counter method was also used in one set of experiments. The bacteria were exposed to ampicillin/sulbactam for 2 hr, 24 hr, 72 hr or 7–10 days. Five concentrations, 5, 10, 50 or 100 μg/ml, of the drug were tested. Afterward, the bacteria were washed free of Unasyn® and allowed to multiply. Treatment of the mycobacteria for 2 hr did not produce any PAE, although 100 μg/ml of the drug caused slower growth. Exposure to 50, 60, or 100 μg/ml, resulted in a prolonged PAE of ~13 days. The data on the PAE of Unasyn® may be of clinical relevance in determining dosage regimens of the drug.—Authors' Abstract

Randhawa, B., Harris, E. B. and Prabhakaran, K. Bactericidal action of oral ampicillin/sulbactam against *Mycobacterium leprae.* J. Antimicrob. Chemother. **44** (1999) 279–281.

We reported previously that an injectable form of ampicillin/sulbactam, Unasyn, was bactericidal to *Mycobacterium leprae* multiplying in mouse foot pads. In this study, we examined the effect of an orally active form of ampicillin/sulbactam, Sultamicillin, on the growth of *M. leprae* in mice. Three concentrations of the drug, mixed with the feed, were administered from the start until the mice were killed at 6 months; 0.01% of the drug inhibited bacterial growth by 54%, 0.10% by 74% and 0.20% by 93%. To test whether oral ampicillin/sulbactam was bactericidal, 0.50% of the drug, mixed with the feed, was administered to experimentally infected mice for 3 months during the logarithmic phase of bacterial growth, and then discontinued; multiplication of the bacilli was monitored monthly for the next 8 months. The results showed that orally active ampicillin/sulbactam is bactericidal to *M. leprae.*—Authors' Abstract

Reynolds, R. C., Bansal, N., Rose, J., Fredrich, J., Suling, W. J. and Maddry, J. A. Ethambutol-sugar hybrids as potential inhibitors of mycobacterial cell-wall biosynthesis. Carbohydr. Res. **317** (1999) 164–179.

Ethambutol is an established front-line agent for the treatment of tuberculosis, and is also active against *Mycobacterium avium* infection. However, this agent exhibits toxicity, and is considered to have low potency.

The action of ethambutol on the mycobacterial cell wall, particularly the arabinan, and comparison of the structure of ethambutol with several of the cell-wall saccharides, suggested that ethambutol-saccharide hybrids might lead to agents with a more selective mechanism of action. To this end, eight ethambutol-saccharide hybrids were synthesized and screened against *M. tuberculosis* and several clinical isolates of *M. avium.*—Authors' Abstract

Steel, H. C., Matlola, N. M. and Anderson, R. Inhibition of potassium transport and growth of mycobacteria exposed to clofazimine and B669 is associated with a calcium-independent increase in microbial phospholipase A^2 activity. J. Antimicrob. Chemother. **44** (1999) 209–216.

Altered phospholipase A^2 (PLA^2) activity and its relationship to cation (K+, Ca2+) uptake and growth were investigated in mycobacteria exposed to the riminophenazine antimicrobial agents, clofazimine and B669

(0.15–2.5 mg/L). Microbial PLA activity was measured using a radiometric thin-layer chromatography procedure; whereas K+ and Ca2+ transport were measured using Rb86+ or K-42+ and Ca-45(2+), respectively. Short-term exposure (15–30 min) of *Mycobacterium aurum,* A+ or the virulent and avirulent isolates of *M. tuberculosis* H37R to the riminophenazines resulted in dose-related enhancement of microbial PLA activity, which was associated with inhibition of K+ influx and growth. Uptake of Ca2+ by mycobacteria was unaffected, or minimally affected, by the riminophenazines at concentrations of less than or equal to 0.6 mg/L; whereas higher concentrations resulted in increased uptake of the cation in the setting of decreased microbial ATP concentrations. The results of kinetic studies using a fixed concentration (2.5 mg/L) of B669 demonstrated that riminophenazine-mediated enhancement of PLA activity and inhibition of K+ uptake in mycobacteria are rapid and probably related events that precede, by several minutes, any detectable effects on microbial ATP concentrations and uptake of Ca2+. Inclusion of the extracellular and intracellular Ca2+-chelating agents EGTA (0.2–7.2 g/L) and BAPTA/FURA-2 (0.2–9.5 mg/L), individually or in combination, did not prevent the effects of B669 on mycobacterial PLA, activity or K+ transport; whereas a-tocopherol, which neutralizes PLA^2 primary hydrolysis products, antagonized the inhibitory effects of the riminophenazines on microbial K+ uptake and growth. These results demonstrate that the antimycobacterial activities of clofazamine and B669 are related to a Ca2+-independent increase in mycobacterial PLA^2, leading to interference with microbial K+ transport.—Authors' Abstract

Clinical Sciences

Choudhuri, H., Thappa, D. M., Kumar, R. H. and Elangovan, S. Bone changes in leprosy patients with disabilities/deformities (a clinico-radiological correlation). Indian J. Lepr. **71** (1999) 203–215.

One-hundred-ten leprosy patients (96 males and 14 females, mean age 45.3 years) with disabilities/deformities were examined radiologically to evaluate bone changes and correlating them with clinical parameters. Most patients (98) had paucibacillary leprosy. The mean duration of leprosy was 7.4 years and that of deformity was 4.1 years. Ten patients presented with reaction. Seventy-five (68.2%) patients had received a full course of antileprosy treatment. The overall prevalence of bone changes was 87.3% (96 patients); specific, nonspecific, osteoporotic and facial changes were seen in 44.5%, 75.5%, 38.2% and 9.1% of the patients, respectively. Among the specific bone changes, primary periosteitis (28.2%) and "bone cysts" (22.7%) were the more common findings. Among the nonspecific bone changes, terminal phalangeal absorption (48.2%), soft tissue changes (44.5%) and concentric absorption (32.7%) were more common. Specific bone changes showed a significant ($p < 0.05$) increase with lack of or incomplete antileprosy treatment. Nonspecific bone changes showed significant correlation ($p < 0.05$) with increasing duration of disease, lack of or partial treatment and rising disability index. Osteoporotic changes showed a significant relationship with rising disability index.—Authors' Abstract

Eisig, J. N., Zaterka, S., Boyd, H. K., Marchese, L. C. T. M. and Laudanna, A. A. Hansen's disease and the digestive system: clinical symptoms and gastric secretory profile at baseline conditions and following maximum stimulation with pentagastrin. Acta Leprol. **11** (1999) 99–104.

The incidence of digestive symptoms in 100 patients with Hansen's disease was evaluated in this study, following a standardized questionnaire. A correlation between the frequency of symptoms, the form

of the disease, and the length of treatment was investigated. Digestive symptoms were found in 31 patients (31%). No statistically significant difference was found between the presence of symptoms and the length of the disease or between the multibacillary (MB) and the paucibacillary (PB) form of the disease. However, a positive correlation between digestive symptoms and Hansen's disease was found in the MB form of the disease only for patients treated for more than 12 months. Baseline and pentagastrin-stimulated gastric acid secretion was studied in 30 Hansen's disease patients and in 10 controls. A lower basal acid output was observed in patients with Hansen's disease, but no statistical difference was found. Pentagastrin-stimulated gastric acid secretion was statistically different in Hansen's disease patients, as compared to controls. A lower pentagastrin-stimulated acid secretion was found in Hansen's disease patients under treatment, as compared to untreated patients, but the difference was not statistically significant.—Authors' Summary

Indira, D., Kaur, I., Sharma, V. K. and Das, A. Palmoplantar lesions in leprosy. Indian J. Lepr. **71** (1999) 167–172.

Palms and soles are considered immune to leprosy. A study was carried out to assess the frequency of lesions over palms and soles and to correlate their occurrence with various parameters. Two-hundred-eighty leprosy patients were screened for lesions over palms and soles. Palmo-plantar lesions were observed in 10% of the patients screened. Slit-skin smears and biopsies were done from routine sites and palmoplantar lesions. Histopathology and slit-skin smear confirmed the presence of disease. Eight were in type 1 reaction, and 50% of the patients with type 1 reaction screened showed lesions over palms and/or soles. The reason for this is not known; probably inapparent lesions become apparent during reactions. Lesions of various morphology were observed. Silky hand was observed in one case.—Authors' Abstract

Malik, A., Bhatia, A., Singh, N., Bhattacharya, S. N. and Arora, V. K. Fine needle aspiration cytology of reactions in leprosy. Acta Cytol. **43** (1999) 771–776.

Objective: To define diagnostic cytomorphologic features of reactions in leprosy.

Study design: Part-retrospective, part-prospective, single-blind, controlled study of the applicability of fine needle aspiration cytology in the diagnosis of reactions in leprosy. Cytomorphologic features were compared in 42 clinically diagnosed patients with reactions in leprosy with those in a control group of patients with nonreactional leprosy. The study groups included type 1 and type 2 reactions in 35 and 9 patients, respectively. May-Grunwald-Giemsa and Ziehl-Neelsen staining methods were employed.

Results: Statistically significant ($p < 0.01$) cytomorphologic features of type 1 reaction were the presence of fragments of collagen and elastin; giant cells; giant cells exhibiting elastin phagocytosis; loose, epithelioid cell granulomas; and fibroblasts. Type 2 reaction was characterized in aspirates by the presence of an abundance of neutrophils in a background of lepromatous leprosy.

Conclusion: Criteria that are used in histopathology for the diagnosis of leprosy reactions can be applied satisfactorily to cytologic smears. A good correlation between clinical diagnosis and cytomorphology can be achieved. Multiple-site aspirates from the skin, nerve and lymph nodes are helpful in substantiating the diagnosis.—Authors' Abstract

Srinivasan, S., Nehru, V. I., Mann, S. B. S., Sharma, V. K., Bapuraj, J. R. and Das, A. Study of ethmoid sinus involvement in multibacillary leprosy. J. Layrngol. Otol. **112** (1998) 1038–1041.

In a prospective study, 25 untreated patients with multibacillary leprosy attending the leprosy clinic of Nehru Hospital, PGIMER, Chandigarh, India [date not given] were included. Clinical examination, computed tomography (CT) scan of paranasal sinuses, ethmoid sinus endoscopy and biopsy were carried out in all patients to investigate the involvement of the paranasal sinuses in leprosy. Ethmoid sinus involvement was noted in 20 patients on

CT scan. Bilateral involvement was more common (65%). Anterior ethmoids were more commonly affected (65%). On ethmoid sinus endoscopy abnormal mucosa was noted in 17 patients (68%). Ethmoid sinus biopsy was confirmative in 16 patients (64%). Statistically significant correlation was found between CT findings, sinus endoscopy and sinus biopsy findings.—Trop. Dis. Bull. **96** (1999) 833

Valentini, A., Nery, J. A. C., Salles, A. M., Vieira, L. M. M. and Sarno, E. N. [Edema in leprosy; clinical and therapeutical aspects.] Rev. Soc. Bras. Med. Trop. **32** (1999) 131–138. (in Portuguese)

In a 1-year follow-up study of leprosy patients (10 multibacillary and one paucibacillary) in Brazil who had been submitted to a clinical protocol for diagnosis and pathological classification, a clinical pattern of localized and/or systemic edema was observed. Among these patients, 5 simultaneously presented with other symptoms related to reactional states, 4 were diagnosed as type 1, and 1 as type 2. However, although 3 of the patients did not present with reaction when edema was diagnosed, they did develop some aspects of reactional disease at a later stage (2 had neuritis and 1 had type 1 reaction). The edema that preceded or was associated with reactional episodes showed clinical regression as a result of specific treatment against reactions (corticosteroids and/or pentoxifylline and/or thalidomide) in the absence of any other treatment normally used to treat edema. It is suggested that immunological mechanisms are involved in the physiopathology of edema in leprosy.—Trop. Dis. Bull. **96** (1999) 833

Immuno-Pathology

Balcewicz Sablinska, M. K., Gan, H. X. and Remold, H. G. *Mycobacterium avium* attenuates mycobacteria-induced apoptosis by reduction of TNF-alpha activity. J. Infect. Dis. **180** (1999) 1230–1237.

Normal human macrophages respond to infection with *Mycobacterium avium,* serovar 4, by producing tumor necrosis factor (TNF)-alpha, which mediates apoptosis, and by elaborating interleukin (IL)-10, a TNF-alpha antagonist. We show that IL-10 downregulates apoptosis by inhibiting the TNF-alpha production of the inoculated macrophages and by inducing the release of soluble TNF receptor type 2 from the macrophages, which leads to inactivation of TNF-alpha. These experiments suggest that induction of IL-10 production is a virulence factor that creates an intracellular sanctuary for the bacteria that is inaccessible to the defense mechanisms of the host.—Authors' Abstract

Chakrabarty, A. N., Dastidar, S. G., Chandra, A. K., Mukherjee, M. and Chaudhuri, S. K. A comparative study of the Mitsuda type response to antigens of chemoautotrophic nocardioform bacteria and to standard lepromin in leprosy patients. Acta Leprol. **11** (1999) 105–112.

Anergy, or contrarily, Mitsuda-type responses toward 4 chemoautotrophic nocardioform antigens (CAN-Ags) and a control standard lepromin were tested in 73 LL, TT and borderline cases of leprosy. The antigens injected per patient varied from a maximum of 5 to a minimum of 2. Complete anergy to CAN-Ags was seen in 92/92 instances tested on 24 LL cases. The anergy was weakly modified or unmodified in 3 other LL cases which had been vaccinated before. Concurrent studies with the same antigens tested on 33 TT cases showed clear-cut, dose-dependent, Mitsuda-type late responses in 80/81 instances. The CAN bacteria, therefore, despite their origin from different unrelated leprous human, mouse foot pad (MFP) and armadillo tissues, appeared to be identical with each other and also probably related to the leprosy bacillus, on the basis of these parameters.—Authors' Abstract

Frevel, T., Schafer, K. L., Totsch, M., Bocker, W. and Dockhorn Dworniczak, B. PCR based detection of mycobacteria in paraffin wax embedded material routinely processed for morphological examination. J. Clin. Pathol.-Mol. Pathol. **52** (1999) 283–288.

Background—The incidence of mycobacterial infections has increased during the past 5 years. A prompt diagnosis is indispensable for initiating appropriate treatment. Because culturing of mycobacteria takes 3 to 6 weeks and sensitivity of microscopic detection of acid-fast bacilli is low, amplification methods provide promising possibilities. Recently, the polymerase chain reaction (PCR) has been shown to be useful for confirming a mycobacterial infection, especially in cases with unexpected histological findings or lack of suitable material for culturing.

Aims—To evaluate the impact of PCR-based techniques in the detection of mycobacterial infections in uncultured routine histological specimens as an alternative to surgical pathology.

Methods—Two-hundred-twenty-nine formalin-fixed and paraffin wax-embedded samples from 141 patients with clinical or histological suspicion of a mycobacterial infection were investigated using three different PCR assays and Southern blotting. PCR results were compared with histology and culture and the patients' clinical findings.

Results—When using culture as the reference method, the sensitivity for the detection of mycobacteria of the tuberculosis complex was 90%, specificity was 92%, the positive predictive value was 81%, and the negative predictive value was 96%. The sensitivity for the detection of nontuberculous mycobacteria was 100% and specificity was 78%, the positive predictive value was 26%, and the negative predictive value was 100%. The patients' clinical findings supported the PCR-positive results, indicating a mycobacterial infection in 11 of 18 initially culture-negative cases and in 21 of 35 PCR-positive cases without culture results.

Conclusions—These results indicate that PCR-based techniques are sensitive, specific, and rapid methods for the detection of mycobacteria in routinely processed paraffin wax-embedded and formalin-fixed histological samples.—Authors' Abstract

Gupta, A., Sharma, V. K., Vohra, H. and Ganguly, N. K. Inhibition of apoptosis by ionomycin and zinc in peripheral blood mononuclear cells (PBMC) of leprosy patients. Clin. Exp. Immunol. **117** (1999) 55–62.

PBMC from tuberculoid (BT/TT) and lepromatous leprosy (BL/LL) leprosy patients showed spontaneous apoptosis when cultured in the absence of mitogen for 24 hr, which was inhibited by antitumor necrosis factor-alpha (TNF-α) antibodies. Apoptosis was also inhibited by ionomycin and zinc, which also increased IL-2 and decreased TNF-α production. The increase in IL-2 production suggests a mechanism whereby dietary supplements with zinc might alter the cell-mediated immunity response in leprosy patients.—Authors' Abstract

Hussain, R., Kifayet, A., Dojki, M. and Dockrell, H. M. Selective correlation of interferon-gamma, tumour necrosis factor-alpha and granulocyte-macrophage colony-stimulating factor with immunoglobulin G1 and immunoglobulin G3 subclass antibody in leprosy. Immunology **98** (1999) 238–243.

Dysregulation of both B- and T-cell responses is observed in leprosy. Immunoglobulin G1 (IgG1) and IgG3 antibody subclasses are selectively elevated toward the lepromatous or disseminated form of the disease accompanied by a depression of T-cell responses. T-cell and macrophage cytokines influence antibody class switching, differentiation and proliferation of B cells. To understand the dynamic nature of the immune response in leprosy, we examined the relationship between circulating *Mycobacterium leprae*-specific antibodies and secreted cytokines [interferon-gamma (IFN-γ), interleukin-2 (IL-2), IL-5, IL-10, IL-6, tumor necrosis factor-alpha (TNF-α) and granulocyte-macrophage colony-stimulating factor (GM-CSF)] in leprosy patients (19 lepromatous patients; 25 tuberculoid patients) and their exposed household con-

tacts (HC = 14) in response to *M. leprae* antigens. Paired comparison revealed a highly significant negative correlation between IFN-γ and IgG ($p = 0.016$), IgG1 ($p < 0.001$) and IgG3 ($p = 0.007$) antibodies. No significant relationship was observed with other T-cell cytokines (IL-2, IL-5 and IL-10). These results strongly suggest that IFN-γ may play a role in downregulating antigen-specific IgG1 and IgG3 antibodies. Among the macrophage cytokines, TNF-γ and GM-CSF which have not been shown to play a role in B-cell activation were positively associated with IgG1 (TNF-α, $p = 0.0005$; GM-CSF, $p = 0.001$) and IgG3 (TNF-α, $p = 0.001$; GM-CSF, $p = 0.021$) antibodies. Since macrophages have high-affinity Fc receptors for IgG1 and IgG3, it is possible that antigen uptake via these receptors may influence cytokine expression of TNF-α, a key modulator of disease pathogenesis in mycobacterial diseases. We are currently investigating the role of Fc receptors on activated macrophages, in expression of pro-inflammatory cytokines in mycobacterial diseases.—Authors' Abstract

Lugton, I. W. Mucosa-associated lymphoid tissues as sites for uptake, carriage and excretion of tubercle bacilli and other pathogenic mycobacteria. Immunol. Cell Biol. **77** (1999) 364–372.

Pathogenic mycobacteria, including those that cause tuberculosis and paratuberculosis, cross mucosal barriers by endocytosis within mucosal lympho-epithelial sites. These entry sites commonly include oropharyngeal and nasopharyngeal tonsils and Peyer's patches. Bacilli discharged at the basolateral surfaces of engulfing epithelial M cells are taken up by professional antigen-presenting cells associated with T lymphocytes of the parafollicular area. Dendritic cells and macrophages in these sites allow mycobacterial replication, due to the permissive immunological environment in lymphoepithelial tissues. Abrogation of local delayed-type hypersensitivity reactions generally ensures continuing integrity and function of these tissues. Phagocytes containing intracellular mycobacteria disseminate infection to other parts of the body and also probably migrate back onto the mucosal surface to shed bacilli.—Author's Abstract

Maeurer, M., Seliger, B., Trinder, P., Gerdes, J. and Seitzer, U. Interleukin-15 in mycobacterial infection of antigen-presenting cells. Scand. J. Immunol. **50** (1999) 280–288.

Interleukin-15 (IL-15) shares many biological functions with IL-2 but also exhibits unique effects. Some of these represent the potent chemoattractant activity and expansion of distinct T-cell subsets, particularly memory T cells. IL-15 may therefore modulate the quality and quantity of cellular immune responses directed against intracellular pathogens. Immunohistochemical examination of skin lesions obtained from patients with the lepromatous or the tuberculoid form of Hansen's diseases revealed intralesional IL-15 protein in both forms of the disease. In addition to *Mycobacterium leprae,* a number of different mycobacterial species are capable of effectively inducing IL-15 secretion in infected macrophages. In this work, increased IL-15 secretion was observed in IL-4/granulocyte-macrophage colony-stimulating factor (GM-CSF)-activated antigen-presenting cells (APC) compared with unstimulated macrophages. Immunocytological detection of intracellular IL-15 revealed that infection with different mycobacterial species resulted in different staining patterns of anti-IL-15 immunoreactive material in APC. In contrast to IL-2 or IL-7, IL-15 enhanced the cytolytic potential of immune effector cells *in vitro* and favored the expansion of CD1b-restricted immune cells recognizing mycobacterial-associated antigens presented by autologous APC. IL-15 produced by infected cells *in situ* may represent one of the key cytokines involved in granuloma formation and may aid the augmentation of cellular immune responses directed against mycobacterial-infected cells.—Authors' Abstract

Moubasher, A. El-D. A., Kamel, N. A., Zedan, H. and Raheem, D. El-D. A. Cytokines in leprosy, I. Serum cytokine profile in leprosy. Int. J. Dermatol. **37** (1998) 733–740. (68 ref.)

Serum levels of interferon-gamma (IFN-γ), interleukin-2 (IL-2), interleukin-2 receptor (IL-2R), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) were measured by ELISA in 55 untreated leprosy patients and 35 reactional leprosy patients from Egypt, in addition to 20 age- and sex-matched healthy controls. Leprosy patients showed significantly higher serum levels of the studied cytokines (except IL-2) compared with healthy controls. When the 2 poles were compared, tuberculoid leprosy (TT) patients showed significantly higher levels of IFN-γ and TNF-α with significant negative correlations with the bacterial index (BI); whereas lepromatous leprosy (LL) patients showed significantly higher serum levels of IL-2R, IL-10 and IL-1β with significant positive correlations with the BI. Both type 1 and type 2 reactional patients showed significantly higher serum IFN-γ, IL-2R and IL-1β, in addition to IL-10 in type 2 reactional patients, compared with nonreactional leprosy patients. When compared with each other, type 1 reactional patients showed increased levels of IFN-γ; whereas type 2 reactional patients showed increased levels of IL-10. It is concluded that in leprosy patients, both IFN-γ and TNF-α are immunoprotective; whereas IL-2R, IL-10 and IL-1β are immunosuppressive. These results indicated that type 1 reaction, with increased levels of IFN-γ, is a cell-mediated immune response; whereas type 2 reaction, with increased levels of IL-10, is essentially an immune complex disease.—Trop. Dis. Bull. **96** (1999) 833–834

Moubasher, A. El-D. A., Kamel, N. A., Zedan, H. and Raheem, D. El-D. A. Cytokines in leprosy, II. Effect of treatment on serum cytokines in leprosy. Int. J. Dermatol. **37** (1998) 741–746. (41 ref.)

The effect of treatment on serum cytokines was evaluated in 36 leprosy patients and 35 reactional leprosy patients from Egypt, and compared with that in 20 age- and sex-matched healthy individuals. The ELISA technique was used to measure serum levels of interleukin-2 receptor (IL-2R), interleukin-10 (IL-10) and interleukin-1beta (IL-1β) before and after treatment. These cytokines represent T-helper 1 (TH1), T-helper 2 (TH2), and macrophage cytokines, respectively. The studied serum cytokines were significantly reduced after 1 year of treatment in leprosy patients. The degrees of reduction were significantly positively correlated with a reduction in the bacterial index (BI) and morphological index (MI). After 1 year of multidrug therapy (but not 6 months), paucibacillary (PB) patients showed a significant reduction in all the studied serum cytokines to levels comparable with those of healthy controls. Multibacillary (MB) patients also showed a significantly reduction in all the studied serum cytokines, but the levels were still significantly higher than those of healthy controls. Leprosy patients with high levels of serum IL-1β were more susceptible to the development of reactions after the initiation of treatment. Corticosteroid therapy of reactional patients resulted in a significant reduction in the studied serum cytokines to levels similar or lower than those of nonreactional leprosy patients. The dose of steroids showed a significant positive correlation with the amount of decrease in IL-1β. MDT caused a reduction in serum cytokines correlated with a reduction in the bacterial burden. It is advisable to continue MDT for PB patients for 1 year. Serum IL-1β levels may have a prognostic value for the susceptibility of leprosy patients to the development of reactions.—Trop. Dis. Bull. **96** (1999) 834

Munk, M. E., Anding, P., Schettini, A. P. M., Cunha, M. da G. S. and Kaufmann, S. H. E. Soluble tumor necrosis factor alpha receptors in sera from leprosy patients. Infect. Immun. **67** (1999) 423–425.

Serum levels of soluble tumor necrosis factor alpha receptor I (sTNF-RI) were elevated in patients from Amazonas State, Brazil, who had lepromatous (LL) reactional-state type 2 leprosy, and sTNF-RII levels were increased in patients with full tuberculoid (TT) or LL type 2 leprosy. The sTNF-R in sera from patients with type 2 leprosy, but not other forms of leprosy, in-

hibited recombinant TNF cytolytic activities *in vitro*. It is suggested that sTNF-R regulatory activities are partially impaired in patients with leprosy.—Trop. Dis. Bull. **96** (1999) 712

Natrajan, M., Katoch, K. and Katoch, V. M. Histology and immuno-histology of lesions clinically suspicious of leprosy. Acta Leprol. **11** (1999) 93–98.

Forty-six patients presenting with lesions clinically suspicious of leprosy were selected among patients attending the outpatient department (OPD) of our Institute. The lesions were biopsied deeply for histological analysis. The cases chosen commonly belonged to the 20–35 years age group, were predominantly males, with rare cases of leprosy within the family (2/46, 4.35%). The clinical presentation in most of the cases was that of a solitary lesion, (44/46, 95.65%) located in one of the extremities (40/46, 86.95%). A positive Mitsuda reaction could be elicited in 40% of the cases. Routine histopathologic analysis, using defined criteria, has established the diagnosis of leprosy in 16/46 (34.78%) cases with positivity for acid-fast bacilli in 4 cases. The remaining cases (25/46, 54.35%) exhibited a nonspecific histopathology with a perivascular/periadnexal mononuclear cell infiltrate, few (5/46, 10.86%) exhibited minimal or no histopathological features. The sections with nonspecific or minimal pathology when immunostained for the presence of mycobacterial antigen exhibited positivity in 11/30 (36.6%) cases. Presuming the features observed to be consequent to the presence of antigens nearby, the diagnosis of leprosy was significantly enhanced compared to the diagnosis achieved with routine histopathology alone.—Authors' Abstract

Rafi, A. IgG response to some mycobacterial antigens in selected leprosy patients. SE Asian J. Trop. Med. Public Health **29** (1998) 567–571.

Serum samples from leprosy patients in Iran with putative tuberculosis were tested by indirect ELISA to determine the level of IgG antibody against 6 mycobacterial antigen preparations. PCR-positive leprosy patients were confirmed with an ELISA based on phenolic glycolipid-I, a prominent capsular lipid from *Mycobacterium leprae*. It is suggested that the ratio of antibodies to *M. tuberculosis* and *M. leprae* antigens is a valuable serological marker for tuberculosis in long-treated leprosy patients.—Trop. Dis. Bull. **96** (1999) 823

Shimoji, Y., Ng, V., Matsumura, K., Fischetti, V. A. and Rambukkana, A. A 21-kDa surface protein of *Mycobacterium leprae* binds peripheral nerve laminin-2 and mediates Schwann cell invasion. Proc. Natl. Acad. Sci. U.S.A. **96** (1999) 9857–9862.

Nerve damage is the hallmark of *Mycobacterium leprae* infection, which results from *M. leprae* invasion of the Schwann cell of the peripheral nervous system. We have recently shown that the laminin-2 isoform, especially the G domain of laminin alpha 2 chain, on the Schwann cell-axon unit serves as an initial neural target for *M. leprae*. However, *M. leprae* surface molecules that mediate bacterial invasion of peripheral nerves are entirely unknown. By using human alpha 2 laminins as a probe, a major 28-kDa protein in the *M. leprae* cell wall fraction that binds alpha 2 laminins was identified. After N-terminal amino acid sequence analysis, PCR-based strategy was used to clone the gene that encodes this protein. Deduced amino acid sequence of this *M. leprae* laminin-binding protein predicts a 21-kDa molecule ML-LBP21, which is smaller than the observed molecular size in SDS/PAGE. Immunofluorescence and immunoelectron microscopy on intact *M. leprae* with mAbs against recombinant (r) ML-LBP21 revealed that the protein is surface exposed. rML-ML-LBP21 avidly bound to alpha 2 laminins, the rG domain of the laminin-alpha 2 chain, and the native peripheral nerve laminin-2. The role of ML-LBP21 in Schwann cell adhesion and invasion was investigated by using fluorescent polystyrene beads coated with rML-LBP21. Although beads coated with rML-LBP21 alone specifically adhered to and were ingested by primary Schwann cells, these functions were significantly enhanced when beads were preincubated with exogenous alpha 2 laminins. Taken together, the pres-

ent data suggest that ML-LBP21 may function as a critical surface adhesin that facilitates the entry of *M. leprae* into Schwann cells.—Authors' Abstract

Smith, S. M., Malin, A. S., Lukey, P. T., Atkinson, S. E., Content, J., Huygen, K. and Dockrell, H. M. Characterization of human *Mycobacterium bovis* bacille Calmette-Guerin-reactive CD8+ T cells. Infect. Immun. **67** (1999) 5223–5230.

Gamma interferon (IFN-γ)-secreting CD4+ T cells have long been established as an essential component of the protective immune response against *Mycobacterium tuberculosis.* It is now becoming evident from studies with the murine model of tuberculosis that an important role also exists for major histocompatibility complex (MHC) class I-restricted CD8+ T cells. These cells are capable of acting as both IFN-γ secretors and cytotoxic T lymphocyte (CTL) effecters; however, their exact role in immunity against tuberculosis remains unclear. This study demonstrates the presence of *M. bovis* BCG-reactive CD8+ T cells in healthy BCG-vaccinated donors and that these CD8+ T cells are potent cytokine producers as well as cytotoxic effector cells. Using FACScan analysis, we have shown that restimulation with live *M. bovis* BCG induced more CD8+-T-cell activation than the soluble antigen purified protein derivative and that these cells are actively producing the type 1 cytokines IFN-γ and tumor necrosis factor alpha (TNF-α). These CD8+ T cells also contain the cytolytic granule perforin and are capable of acting as potent CTLs against *M. bovis* BCG-infected macrophages. The mycobacterial antigens 85A and B (Ag85A and Ag85B, respectively), and to a lesser extent the 19- and 38-kDa proteins, are major antigenic targets for these mycobacterium-specific CD8+ T cells, while whole-*M. bovis* BCG activated effector cells from these BCG-vaccinated donors, as expected, failed to recognize the 6-kDa ESAT-6 protein. The use of metabolic inhibitors and blocking antibodies revealed that the CD8+ T cells recognize antigen processed and presented via the classical MHC class I pathway. These data suggest that CD8+ T cells may play a critical role in the human immune response to tuberculosis infection.—Authors' Abstract

Spierings, E., de Vlieger, M., Brand, A., Klatser, P. R. and Ottenhoff, T. H. M. Antibodies to sulfatide in leprosy and leprosy reactions. Am. J. Trop. Med. Hyg. **61** (1999) 495–499.

Antibodies to sulfatide have been reported in various demyelinating peripheral polyneuropathies. We have investigated the diagnostic value of these antibodies in leprosy. Antisulfatide IgM in leprosy patients was not significantly elevated. High antisulfatide IgG titers were observed in individuals from endemic areas, irrespective of their leprosy status, while Western European controls were negative. No significant correlation was found between IgM or IgG antibody titers and leprosy classification, although multibacillary patients had higher antisulfatide IgM titers than paucibacillary patients. In addition, 23 patients developing leprosy reactions were followed longitudinally. Antibody titers in these patients fluctuated slightly during the follow-up period. There was no associated with the occurrence of leprosy reactions or treatment. Thus, IgG titers against sulfatides are high in both leprosy patients and healthy controls in endemic areas; whereas such antibodies are not found in Western European controls, suggesting that these antibodies are induced by environmental factors, such as microorganisms.—Authors' Abstract

Stewart, G. R., Boussinesq, M., Coulson, T., Elson, L., Nutman, T. and Bradley, J. E. Onchocerciasis modulates the immune response to mycobacterial antigens. Clin. Exp. Immunol. **117** (1999) 517–523.

Chronic helminth infection induces a type-2 cellular immune response. In contrast to this, mycobacterial infections commonly induce a type-1 immune response which is considered protective. Type-2 responses and diminished type-1 responses to mycobacteria have been previously correlated with active infection states such as pulmonary tuberculosis and lepromatous leprosy. The present study examines the im-

mune responses of children exposed to both the helminth parasite *Onchocerca volvulus* and the mycobacterial infections, *Mycobacterium tuberculosis* and *M. leprae*. Proliferation of peripheral blood mononuclear cells (PBMC) and production of IL-4 in response to both helminth and mycobacterial antigen (PPD) decreased dramatically with increasing microfilarial (MF) density. Although interferon-gamma (IFN-γ) production strongly correlated with cellular proliferation, it was surprisingly not related to MF density for either antigen. IL-4 production in response to helminth antigen and PPD increased with ascending children's age. IFN-γ and cellular proliferation to PPD were not related to age, but in response to helminth antigen wee significantly higher in children of age 9–12 years than children of either the younger age group (5–8 years) or the older group (13–16 years). Thus, there was a MF density-related downregulation of cellular responsiveness and age-related skewing toward type 2 which was paralleled in response to both the helminth antigen and PPD. This parasite-induced immunomodulation of the response to mycobacteria correlates with a previous report of doubled incidence of lepromatous leprosy in onchocerciasis hyperendemic regions. Moreover, this demonstration that helminth infection in humans can modulate the immune response to a concurrent infection or immunological challenge is of critical importance to future vaccination strategies.—Authors' Abstract

Uko, G. P., Lu, L. Y., Asuquo, M. A., Fici, D., Mahan, S., Awdeh, Z., Udim, E. R., Ding, W., Umana, U., Adewole, T. and Fraser, P. A. HLA-DRB1 leprogenic motifs in Nigerian population groups. Clin. Exp. Immunol. **118** (1999) 56–62.

Amino acid residues involved in the peptide binding groove of HLA-DRB1 alleles were examined in three Nigerian ethnic groups with leprosy (N = 287) and 170 controls to determine the role of DRB1 alleles in disease outcome with *Mycobacterium leprae*. Nine positively charged motifs and two others with neutral charge to the binding groove were detected. These motifs occurred more frequently in leprosy (leprogenic) than was expected by chance ($p < 0.0001$). In contrast, five motifs with net negative or "modified" neutral charges to the pocket were negatively associated with leprosy. We conclude that clinical outcome of infection with *M. leprae* is largely determined by a shared epitope in DRB1 alleles marked by several motifs. These motifs occur in otherwise normal DRB1 alleles, characterized by net positive or neutral charges in the binding groove. We hypothesize that these polarities cause poor binding of DRB1 to *M. leprae*. On presentation, the signal via the T-cell receptor results in muted cell-mediated immunity. The resulting response translates to various forms of leprosy depending on degree of charge consonance between *M. leprae* and host DRB1 allele. Other factors within or without the HLA complex, such as the T-cell receptor repertoire, may also influence the resulting disease.—Authors' Abstract

Wilkinson, K. A., Katoch, K., Sengupta, U., Singh, M., Sarin, K. K., Ivanyi, J. and Wilkinson, R. J. Immune responses to recombinant proteins of *Mycobacterium leprae*. J. Infect. Dis. **179** (1999) 1034–1037.

A total of 30 individuals were studied in India (22 with leprosy and 8 healthy controls who were heavily exposed but disease-free) by assaying the proliferative, interferon (IFN)-γ, and antibody responses to recombinant antigens of *Mycobacterium leprae* (10, 28, 36, and 65 kDa). The 10-kDa antigen elicited IFN-γ production from all tuberculoid (TT) and borderline tuberculoid (BT) patients but little from controls, lepromatous (LL), or borderline lepromatous (BL) patients ($p < 0.05$). Production of 65-kDa-specific IFN-γ was higher in TT/BT than in controls or LL/BL patients ($p < 0.006$). All subjects produced 65-kDa-specific antibody, but it was higher in LL/BL patients than in healthy controls, whose responses were higher than in TT/BT subjects ($p = 0.035$). The 36-kDa antibody responses were selectively increased in LL/BL subjects ($p < 0.02$). It is suggested that the intermediate phenotype of the controls indicates that *M. leprae*-specific production of IFN-γ may contribute to pathology and to protection in leprosy.—Trop. Dis. Bull. **96** (1999) 833

Microbiology

Flesselles, B., Anand, N. N., Remani, J., Loosmore, S. M. and Klein, M. H. Disruption of the mycobacterial cell entry gene of *Mycobacterium bovis* BCG results in a mutant that exhibits a reduced invasiveness for epithelial cells. FEMS Microbiol. Lett. **177** (1999) 237–242.

Mycobacteria belonging to the *Mycobacterium tuberculosis* complex have the ability to invade and replicate in nonphagocytic cells, an event that requires the presence of bacterial surface components capable of triggering a cell response and the subsequent internalization of the microorganism. In this study, we report the sequencing of the mycobacterial cell entry gene (mce) of *M. bovis* bacillus Calmette-Guerin (BCG) and the generation and characterization of a mutant BCG strain with an inactivated mce gene, by homologous recombination with double cross-over. This mutant strain does not express the mycobacterial cell entry protein (Mce) and exhibits a reduced ability to invade the nonphagocytic epithelial cell line HeLa as compared to wild-type BCG.—Authors' Abstract

Ghanekar, K., McBride, A., Dellagostin, O., Thorne, S., Mooney, R. and McFadden, J. Stimulation of transposition of the *Mycobacterium tuberculosis* insertion sequence IS*6110* by exposure to a microaerobic environment. Mol. Microbiol. **33** (1999) 982–993.

The *Mycobacterium tuberculosis*-specific insertion sequence IS*6110/986* has been widely used as a probe because of the multiple polymorphism observed among different strains. To investigate transposition of IS*6110*, a series of artificially constructed composite transposons containing IS*6110* and a kanamycin resistance marker were constructed. The composite transposons were inserted into a conditionally replicating, thermosensitive, *Escherichia coli*-mycobacterial shuttle vector and introduced into *M. smegmatis* mc^2155. Lawns of transformants were grown at the permissive temperature on kanamycin-supplemented agar and subsequently prevented from further growth by shifting to the non-permissive temperature. Under normal atmospheric conditions, kanamycin-resistant papillae appeared after only about 5–6 weeks of incubation. However, these events were not associated with transposon mobilization. In contrast, lawns that were exposed to a 48 hr microaerobic shock generated kanamycin-resistant papillae after only 6–14 days. These events were generated by conservative transposition of the IS*6110* composite transposon into the *M. smegmatis* chromosome, with loss of the shuttle vector. In common with other IS*3* family elements, transposition of IS*6110* is thought to be controlled by translational frameshifting. However, we were unable to detect any significant frameshifting within the putative frameshifting site of IS*6110*, and the level of frameshifting was not affected by microaerobic incubation. The finding that transposition of IS*6110* is stimulated by incubation at reduced oxygen tensions may be relevant to transposition of IS*6110* in *M. tuberculosis* harbored within TB lesions.—Authors' Summary

Hutter, B. and Dick, T. Upregulation of narX, encoding a putative "fused nitrate reductase" in anaerobic dormant *Mycobacterium bovis* BCG. FEMS Microbiol. Lett. **178** (1999) 63–69.

Mycobacterium tuberculosis and its closely related but nonpathogenic relative *M. bovis* Bacille Calmette-Guerin (BCG) have the capability to adapt to anaerobiosis by shifting down from aerobic growth to a state of nonreplicating persistence or dormancy. Here we report the results of a comparative Northern analysis of 23 genes identified in the tubercle bacillus genome project that might play a role in the energy metabolism under anaerobic conditions. The expression of a majority of the genes was found to be downregulated in the dormant BCG culture. However, the mRNA level for narX, a putative "fused nitrate reductase" not found in other bacteria, was strongly upregulated in anaerobic dormant bacilli. NarX is the first transcriptionally in-

duced gene in anaerobic dormant mycobacteria and might be a useful marker for monitoring the dormancy response in infected animals.—Authors' Abstract

Jungblut, P. R., Schaible, U. E., Mollenkopf, H.-J., Zimny-Arndt, U., Raupach, B., Mattow, J., Halada, P., Lamer, S., Hagens, K. and Kaufmann, S. H. E. Comparative proteome analysis of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG strains: towards functional genomics of microbial pathogens. Mol. Microbiol. **33** (1999) 1103–1117.

In 1993, the WHO declared tuberculosis a global emergency on the basis that there are 8 million new cases per year. The complete genome of the strain H37Rv of the causative microorganism, *Mycobacterium tuberculosis,* comprising 3924 genes has been sequenced. We compared the proteomes of two nonvirulent vaccine strains of *M. bovis* BCG (Chicago and Copenhagen) with two virulent strains of *M. tuberculosis* (H37Rv and Erdman) to identify protein candidates of value for the development of vaccines, diagnostics and therapeutics. The mycobacterial strains were analyzed by two-dimensional electrophoresis (2-DE) combining non-equilibrium pH gradient electrophoresis (NEPHGE) with SDS-PAGE. Distinct and characteristic proteins were identified by mass spectrometry and introduced into a dynamic 2-DE database (http://www.mpiib-berlin.mpg.de/2D-PAGE). Silver-stained 2-DE patterns of mycobacterial cell proteins or culture supernatants contained 1800 or 800 spots, respectively, from which 263 were identified. Of these, 54 belong to the culture supernatant. Sixteen and 25 proteins differing in intensity or position between *M. tuberculosis* H37Rv and Erdman, and H37Rv and *M. bovis* BCG Chicago, respectively, were identified and categorized into protein classes. It is to be hoped that the availability of the mycobacterial proteome will facilitate the design of novel measures for prevention and therapy of one of the great heath threats, tuberculosis.—Authors' Summary

Kai, M., Matsuoka, M., Nakata, N., Maeda, S., Gidoh, M., Maeda, Y., Hashimoto, K., Kobayashi, K. and Kashiwabara, Y. Diaminodiphenylsulfone resistance of *Mycobacterium leprae* due to mutations in the dihydropteroate synthase gene. FEMS Microbiol. Lett. **177** (1999) 231–235.

The nucleotide sequence analysis of the dihydropteroate synthase (DHPS) gene of six diaminodiphenylsulfone-resistant *Mycobacterium leprae* strains revealed that the mutation was limited at highly conserved amino acid residues 53 or 55. Although the mutation at amino acid residue 55 or its homologous site has been reported in other bacteria, the mutation at residue 53 is the first case in bacteria. This is the first paper which links the mutations in DHPS and sulfonamide resistance in *M. leprae.* This finding is medically and socially relevant, since leprosy is still a big problem in certain regions.—Authors' Abstract

Moran, A. J., Doran, J. L., Wu, J., Treit, J. D., Ekpo, P., Kerr, V. J., Roberts, A. D., Orme, I. M., Galant, S., Ress, S. R. and Nano, F. E. Identification of novel immunogenic *Mycobacterium tuberculosis* peptides that stimulate mononuclear cells from immune donors. FEMS Microbiol. Lett. **177** (1999) 123–130.

Proteins which are secreted or associated with the cell envelope of *Mycobacterium tuberculosis* may contain protective T-cell epitopes. Prior to this study, a recombinant clone bank of enzymatically active *M. tuberculosis*-alkaline phosphatase fusions were screened for immunogenicity in a murine T-cell model. Five of these were selected for further study, and the IFN-gamma secretion and proliferation of human PBMC from purified protein derivative-(PPD)-positive and PPD-negative donors were measured in response to oligopeptides, Mtb-PhoA fusions and one full-length protein. Epitopes from four of the five selected antigens were immunoreactive in the human model and corresponded to cytochrome d ubiquinol oxidase, cytochrome c oxidase subunit II, MTV005.02 and MTV033.08. Thus, this strategy identified novel human immunogenic peptides as possible candidates for a subunit vaccine.—Authors' Abstract

Wiker, H. G., Spierings, E., Kolkman, M. A. B., Ottenhoff, T. H. M. and Harboe, M. The mammalian cell entry operon 1 (Mce1) of *Mycobacterium leprae* and *Mycobacterium tuberculosis.* Microb. Pathogen. **27** (1999) 173–177.

The genome project on *Mycobacterium tuberculosis* H37Rv has revealed four mammalian cell entry (MTmce1–4) operons putatively involved with entry and survival of mycobacteria in host cells. A homologous operon to the MTmce1 operon was identified in cosmid B983 of *M. leprae.* By comparison with *M. tuberculosis,* several mutations, or sequencing errors, were predicted at specific sites causing frame shifts in the MLyrbE1A, MLyrbE1b and MLmce1D genes. Using targeted sequencing, sequence errors were identified. The corrected MLmce1 operon sequence appears to be highly homologous to the Mtmce1 operon, and similarly encodes eight potential genes. Thus, both *M. tuberculosis* and *M. leprae* mce1 operons may be functional and involved in host cell targeting.—Authors' Abstract

Epidemiology and Prevention

Atrio Mourino, N. J. and Carrazana Hernandez, G. B. [Some epidemiological aspects of the incidence and prevalence of leprosy in the province of Camagüey, Cuba, 1996.] Rev. Leprol. Fontilles **22** (1999) 133–143. (in Spanish)

A study was carried out in 1997 in the Province of Camagüey, Cuba, to evaluate the incidence and prevalence of leprosy in the area. The epidemiological indicators used were incidence and its population distribution of clinical forms, early and late diagnostic mode of detection, prevalence and distribution of clinical forms. The results reveal that leprosy is still a health problem in Camagüey in 7 of 13 counties; the prevalence rate was $1.2 \times 10{,}000$; incidence $4.4 \times 100{,}000$; multibacillary forms are predominant (82.9%); early detection is at 62.8% and the highest percentage of new cases who attend a medical clinic is 94.3%.—Authors' English Summary

Barua, S., Wakai, S., Shwe, T. and Umenai, T. Leprosy elimination through integrated basic health services in Myanmar: the role of midwives. Lepr. Rev. **70** (1999) 174–179.

Myanmar is one of the top 16 countries identified by WHO as being hyperendemic for leprosy. Multidrug therapy (MDT) was introduced in 1988 as a vertical program and gradually integrated into the basic health services (BHS), achieving 100% coverage over the registered cases by 1995. To achieve maximum coverage of and benefit for patients, both leprosy vertical staff and BHS staff were trained to implement MDT while performing routine BHS activities. This included a total of 8615 trained midwives who were mobilized for the nationwide leprosy elimination program (LEP). They worked at village level in various parts of the country and were willing and able to carry out basic tasks in leprosy management, such as the implementation of MDT using blister-calendar packs carrying a month's supply of drugs. This study was performed to assess the workload of midwives and their attitude toward LEP. The authors conclude that midwives in Myanmar show high levels of commitment and reliability, which are essential contributing factors to achieve the current goal of leprosy elimination by the year 2000. Along with the present trend of decreasing prevalence rate, leprosy could no longer be considered as a public health problem at the national level by the year 2000 in Myanmar. However, because of its long incubation period, new leprosy patients may arise even after the elimination target is achieved, while many other patients may become disabled. A community-based sustainable approach for the post-elimination phase, after the year 2000, will be essential and the contribution of the midwives may be of considerable importance.—Authors' Summary

Cardona-Castro, N., Ortega-Rodriguez, G. and Agudelo-Florez, P. Evaluation of three *Mycobacterium leprae* monoclonal antibodies in mucus and lymph samples from Ziehl-Neelsen stain negative leprosy patients and their household contacts in an Indian community. Mem. Oswaldo Cruz **93** (1998) 487–490.

Mucus and lymph smears collected from leprosy patients and their household contacts in the Caño Mochuelo Indian Reservation, Casanare, Colombia, were examined with monoclonal antibodies (MAb) against *Mycobacterium leprae.* The study group consisted of 5 borderline leprosy (BB) patients and 4 patients with a lepromatous leprosy (LL) (all of whom were undergoing epidemiological surveillance after treatment) and 44 household contacts (21 contacts for the LL group and 23 contacts for the BB group). The MAbs were reactive with the following *M. leprae* antigens: 65-kDa heat-shock protein, A6; soluble antigen, G7; and complete antigen, E11. All the samples were tested with each of the MAbs using the avidin-biotin-peroxidase technique and 3,3 diaminobenzidine as chromogen. The patients and household contacts studied were all recorded as Ziehl-Neelsen stain negative. The MAb which showed optimal reaction was G7; this MAb permitted good visualization of the.bacilli. Five patients with BB and one with LL were positive for G7. Of the BB patients' household contacts, 9 were positive for G7; 7 of the LL patients' household contacts were positive for the same MAb. It is concluded that MAb G7 permitted the visualization of the complete bacillus and could be used for early diagnosis and follow up of the disease in patients.—Trop. Dis. Bull. **96** (1999) 711–712

Chen, X.-S., Li, W.-Z., Jiang, C. and Ye, G.-Y. Prediction of elimination of leprosy in leprosy endemic areas of China. Indian J. Lepr. **71** (1999) 189–201.

A study was carried out based upon the data from the National System for Leprosy Surveillance and using appropriate mathematical models. The result showed that of 337 counties where the national goal of basic eradication of leprosy had not been reached and in 40 counties where the WHO goal of leprosy elimination had not been achieved in 1996, the detection rates in calendar years followed exponential models with significant goodness-of-fit. In the 67 counties with downward trends of detection rates, the national goal can be met in terms of detection rate in 6% of counties before the year 2000 or 34.4% before the year 2010 or, in terms of prevalence rate, in 31.3% before the year 2010. In the 11 counties with downward trends of the detection rates, the WHO target can be met in 8 to 10 counties within this century when the duration of disease was determined with the WHO definition. If the MB proportion among new cases increased by 10%, the target would be met 1 year later. However, at the same MB proportion, the change of fixed treatment schedules from PB 6 months and MB 2 years to PB 9 months and MB 3 years will cause achievement of the goal to be postponed 2 to 10 years.—Authors' Abstract

Convit, J., Avilan, R., Diaz, D., Ulrich, M., Quiroga, R., Aranzazu, N., Borges, R. and Pinardi, M. E. [Leprosy control in Venezuela after more than 10 decades of development.] Rev. Leprol. Fontilles **22** (1999) 145–162. (in Spanish)

The antileprosy campaign in Venezuela began in the 19th century and is re-enforced in the 20th century. The Ministry of Public Health and Social Welfare is created in 1936, including the Antileprosy Service. The Leprosy Division is created in 1946, and it later becomes the Department of Public Health Dermatology, which today is part of the Institute of Biomedicine. The Department has 31 regional services nationwide.

Supervised MDT treatment is initiated in 1985. The evolution of leprosy is characterized by an increase of detection and prevalence after 1946, with a later decrease during the 1960s, stabilizing during the 1980s at rates around 0.25 (slightly over 500 cases) per 10,000 inhabitants per year. The decrease in prevalence has been maintained, with sharp changes in 1992 and 1995 due to updating of registries.

The level of elimination of leprosy as a public health problem (according to the WHO norms of prevalence rates under

1/10,000 inhabitants) was reached in Venezuela during 1997, when only four (Apure, Barinas, Cojedes and Portugesa) of the 23 states show prevalence rates over the elimination level. Since the elimination level has been reached but the number of new cases and the detection rate remain relatively stable, we propose a more strict definition for classifying a country at the "elimination level" by including, apart from the prevalence rate, data regarding case detection. Cases are predominantly multibacillary. Detection rates per age show a gradual increase parallel to age. Male/female rates remain at around 2. Ten per cent of cases show some degree of disability, even though generally discrete. There is a percentage of cases born abroad higher than this group in the general population. Most cases come from marginal urban areas, even though rates are higher for rural areas.

The Institute of Biomedicine has developed a great research effort in this disease, especially in its immunological aspects.—Authors' English Summary

Croft, R. P., Richardus, J. H., Nicholls, P. G. and Smith, W. C. S. Nerve function impairment in leprosy: design, methodology, and intake status of a prospective cohort study of 2664 new leprosy cases in Bangladesh (The Bangladesh Acute Nerve Damage Study). Lepr. Rev. **70** (1999) 140–159.

The Bangladesh Acute Nerve Damage Study (BANDS) is a prospective cohort study designed to investigate epidemiological, diagnostic, therapeutic and operational aspects of acute nerve function impairment in leprosy. The study is based at a single center in Bangladesh, in an area with a high prevalence of leprosy. The center, Danish Bangladesh Leprosy Mission, has a well-established vertical leprosy control program. In this paper, the study design and methodology are described, together with definitions of nerve function impairment (NFI) used in this and subsequent papers. The study recruited 2664 new leprosy cases in a 12-month period. The male : female ratio is 1.25:1, and 17.61% of the cohort are under 15 years of age. In all, 83.33% of the cohort are paucibacillary (PB) and 16.67% multibacillary (MB). However, the MB rate among males is 19.72%, and among females is 12.85%, despite an equal period of delay to diagnosis; 55% of patients presented for treatment within 12 months of developing symptoms; 6.12% of the total number of cases were smear positive and 36.71% of the MB cases were smear positive; 9.61% of the total number of cases were graded as having World Health Organization (WHO) disability grade 1 and 5.97% had grade 2. Among MB cases, 27.48% had WHO grade 1 disability present and 18.24% had grade 2 present compared with 6.04% and 3.51%, respectively, among PB cases. A total of 11.90% presented with NFI needing treatment (3.38%) and of these, 61 (67.78%) had silent NFI. MB patients had a prevalence of reaction/NFI needing treatment nearly 7 times higher than PB cases (15.32% among MB; 2.30% among PB), and males nearly double that of females (5.67% among males; 2.96% among females). The most commonly affected nerve by function impairment was the posterior tibial (sensory) with 6.46% of nerves affected (9.38% of patients), followed by the ulnar nerve with 3.23% of nerves impaired (5.56% of patients). Future research and publications, building on this foundation, will focus on the following areas: the incidence of NFI and reactive events, the risk factors for developing NFI, and the response to treatment of patients developing acute NFI.—Authors' Summary

Naik, S. S. and Ganapati, R. Impact of MDT on leprosy prevalence as judged by surveys in the "megacity" of Mumbai. Indian J. Lepr. **71** (1999) 217–221.

The analysis of survey results in all locations in Mumbai (Bombay) city identified for comparison indicates that widespread and diligent use of MDT has contributed to a significant reduction in the yield of new cases. It is however true that the overall incidence rate in the state or country as a whole has been showing only a very gradual tendency to decline. It is also true that while the possibility of identifying new cases in surveys is becoming very low in the megacity of Mumbai, a few skin-smear-positive cases are still lurking in the urban community before identification by casual

means or voluntary reporting. Moreover, constant influx of population into the city poses further problems in measuring and interpreting the precise impact of MDT on prevalence and new case detection rates in relation to leprosy.—Authors' Conclusion

Richardus, J. H., Meima, A., Croft, R. P. and Habbema, J. D. F. Case detection, gender and disability in leprosy in Bangladesh: a trend analysis. Lepr. Rev. **70** (1999) 160–173.

A trend analysis is presented of all newly detected leprosy cases over an 18-year period (1979–1996) in a highly leprosy-endemic area of Bangladesh. A total of 23,678 new cases were registered, with an average of 860 new cases per year in the first 12 years, and increasing to around 3000 in 1996. The male : female (M:F) ratio decreased from 2.3 to 1.4. The proportions of newly detected cases with MB leprosy and of newly detected cases with any disability decreased over time. These reductions were more marked in the higher age groups of both sexes. The reduction in disability was primarily attributable to a decline in grade 2 disability. New case detection rates (NCDR) of all leprosy patients per 10,000 general population increased for males from 3 to 6; and form females from 1 to 4, while the NCDR of MB leprosy decreased in males from 1.4 to 0.6, and in females fluctuated around 0.45. The NCDRs of leprosy patients with disabilities showed an initial decrease in the first period, especially in males, but later showed an increase. The NCDR of males with disability was about twice as high as that of females. Finally, female NCDRs in the ages between 15 and 30 were low by comparison with the male NCDRs at the same time. This may be due to the sociocultural characteristics of the Bangladeshi society, with gender differences in exposure, health seeking behavior and opportunities for case detection. Operational changes in the control program have contributed to the changed profile of newly detected cases. This study shows that the application of general population statistics is essential for understanding the dynamics in leprosy control programs under changing operational conditions. Combining case detection figures with such statistics helps to identify population groups that are possibly not benefiting sufficiently from the services provided, and to clarify the dynamics in control programs and the future trends and program requirements.—Authors' Summary

Yao, K. J., Koffi, M., Yao, B. G. and Tra, G. [Profile of Hansen's disease in the sanitary district of Odienné, northwest region of Ivory Coast.] Acta Leprol. **11** (1999) 83–87. (in French)

To determine the characteristics of leprosy in the Sanitary District of Odienné in the northwestern part of Ivory Coast, a retrospective study was made based on patients of 4 years. This study showed that 107 leprosy patients were detected from 1994 to 1997, and all of them benefited from polychemotherapy. Leprosy affected youngsters as well as adults (aged 4 to 95) with a slight feminine prevalence (55%). Patients were detected and put on polychemotherapy about 5 years after the first symptoms had appeared. The paucibacillaires represented 72.5% and were most present with women. Disabilities at detection were of 25.7% on the whole with a higher frequency in the multibacillary group (43.3%) than in the paucibacillary (19%); moreover, level 2 disabilities represented 10.1%, most of them being confined on hands. Finally, the efforts of sensibilization seem having given the opportunity to the majority of patients detected in 1997 to come spontaneously for consultation, just after the first signs of illness. This sensibilization has to be sustained.—Authors' English Summary

Rehabilitation

Kazen, R. O. Ulcer surgery for non-specialist surgeons. Lepr. Rev. **70** (1999) 204–211.

In the majority of cases, plantar ulcers in need of surgical intervention can be treated by very simple procedures. Patients benefit from treatment facilities near to their homes. In the process of integration surgery could be made available to leprosy patients in peripheral health units near their homes by training nonspecialist surgeons in peripheral health units in basic surgical procedures, aiming at ulcer healing as well as preventing reoccurrence of ulcers.—Author's Summary

Mathew, J., Antony, P., Ethiraj, T. and Krishnamurthy, P. Management of simple plantar ulcers by home based self-care. Indian J. Lepr. **71** (1999) 173–187.

Seeking a solution to bring down the prevalence of simple plantar ulcers in the field, Damien Foundation India Trust (DFIT), Chennai, developed a curriculum to teach the field staff of all its projects. The purpose was to make patients self-reliant in the care of their plantar ulcers in their homes. The strategy used was to make patients take care of their ulcers using tools found in their homes and surroundings and become responsible for the care of their limbs. This strategy was implemented in eight projects of DFIT and the program was followed regularly for 1 year. Regular monitoring and evaluation showed that under this strategy the prevalence of plantar ulcers was reduced by about 50%.—Authors' Abstract

Meima, A., Saunderson, P. R., Gebre, S., Desta, K., van Oortmarssen, G. J. and Habbema, J. D. F. Factors associated with impairments in new leprosy patients: the AMFES cohort. Lepr. Rev. **70** (1999) 189–203.

Data on the importance of the delay between onset of symptoms and registration as a risk factor for impairment are sparse. This study investigates the quantitative relationship between this delay, other risk factors and the impairment status in new leprosy patients. It reports on 592 new leprosy patients enrolled in 1988–1992 in the prospective ALERT MDT Field Evaluation Study in central Ethiopia (AMFES). The influence of the risk factors, sex, age, delay, PB/MB classification in relation to BI, and prior dapsone treatment on the impairment status at intake is analyzed. Estimates for the delay are based on patient recall. For the risk factors, odds ratios on impairment and on severity of impairment were calculated using both univariate and multivariate logistic regression. The registration delay was 2 years or more for 44% of new patients. The prevalence of impairment (WHO impairment grades 1 and 2 combined) increased continuously from 36% for new patients with a delay of 0–1 year to 81% for new patients with delays of 4 years or more. This prevalence also increased continuously with age; it rose from 26% in children to 80% for the age group 60 and over. In the multivariate regression, the odds ratios for new patients to be impaired were statistically significant for all delay categories (baseline 1–2 years) and age groups (baseline 15–29 years). No statistically significant differences in odds ratios were observed with respect to sex and PB/MB classification in relation to BI. Overall, 31% of new patients presented with WHO impairment grade 1 and 23% with grade 2. The risk on grade 2 also increased with the registration delay among the impaired new patients. Relatively few impaired males and relatively few impaired MB patients with a BI value of 3 or higher had grade 2 impairment. Registration delay and age are the main risk factors for presentation with impairment. Reduction of delay in central Ethiopia requires re-thinking of control methodologies. The search for ways to reduce delays in diagnosis and treatment should receive high priority in leprosy research and in leprosy control programs.—Authors' Summary

Patond, K. R., Betal, B. D. and Gautam, V. Results of thumb correlation in leprosy using different techniques. Indian J. Lepr. **71** (1999) 155–166.

Forty-four hands of 42 leprosy patients with paralysis of the intrinsic muscles of the hand were treated by opponensplasty using ring finger superficialis (FDS_4) or extensor indicis proprius (EIP). Superficialis tendon of middle finger was also used in these hands for lumbrical replacement by "direct lasso" operation. Low ulnar paralysis with Froment's sign was corrected by transfer of radial half of flexor pollicis longus (FPL) to extensor pollicis longus (EPL). Results of thumb correction were assessed and analyzed in 37 hands of 35 patients. The mean follow-up period was 19 months. Best results were found with transfer of half FPL to EPL. Results of FDS transfer was good in 12 out of 16 manual workers. EIP transfer worked well, but the power of the thumb and patients' satisfaction was less.—Authors' Abstract

Sow, S. O., Tiendrebeogo, A., Lienhardt, C., Soula, G., Fomba, A. and Doumbia, M. [Leprosy as a cause of physical disability in rural and urban areas of Mali.] Sante **8** (1998) 296–302. (in French)

A cross-sectional study of populations from two areas of Mali was carried out in May and June 1996 to assess the extent to which leprosy causes physical disability in Mali. One area was rural (Circle of Bougouni), the other urban (Bamako District). A cluster sampling method was used, with 500 households randomly selected for study in each of the two areas. All members of the selected households were included in the study. For all survey sites, the number of households was proportional to the number of inhabitants. The total study population was 8175, including 172 physically handicapped individuals, 76 in Bamako and 96 in Bougouni. The prevalence of physical handicap was 21 per 1000 inhabitants (25.3 per 1000 in rural areas and 17.3 per 1000 in the city). The difference in the prevalence of physical handicap between the two areas was significant ($p = 0.01$). Leprosy was responsible for 10% of the disabilities. The most common causes of disability other than leprosy were trauma and poliomyelitis. Leprosy mostly caused disabilities in rural areas. In both areas, leprosy caused more disabilities in men and boys (64% of cases) than in women and girls. The frequency of disabilities caused by leprosy increased with age; whereas the frequency of handicaps with other causes decreased with age. It is concluded that leprosy is still a major cause of disability in countries in which it is endemic.—Trop. Dis. Bull. **96** (1999) 712

van Brakel, W. H., Reed, N. K. and Reed, D. S. Grading impairment in leprosy. Lepr. Rev. **70** (1999) 180–188.

The aim of the paper is to discuss the concept of "severity grading" in relation to impairment in leprosy, and to describe the use of an impairment sum score, the Eyes, Hands, Feet (EHF) score, as an indicator of the severity and the evolution of impairment over time. The use of an impairment sum score, the EHF score, is illustrated using data on impairment at diagnosis and after a 2-year interval from MB patients released from MDT in the Western Region of Nepal. The WHO 1988 "disability" grading scale (0–2, for both eyes, hands and feet—six sites) was used as a measure of impairment. For the analysis, the WHO grades for the six sites were summed to form an EHF score (minimum 0, maximum 12). The sensitivity to change over time of the EHF score was compared with that of the "method of maximum grades." Using the "method of maximum grades," 509/706 patients (72%) appeared not to have changed in impairment status, compared with only 399 (56.5%) with the EHF score. Improvement or deterioration of impairment status was missed in 113 patients (16%). In 216/706 patients (30.6%), the changes detected with the EHF score were bigger than those revealed by the method of maximum grades. The six components of the WHO impairment grading may be added up to form an EHF sum score of impairment. This score can be used to monitor changes in impairment status in individuals or in groups. It should be recorded and reported at least at diagnosis and release from treatment. Reporting could be done as the "proportion of patients with improved EHF

score," "stable EHF score" and "EHF score worse," and "proportion of patients without impairment," "proportion with WHO grade 1" and "proportion with WHO grade 2." It is recommended that the concepts and terminology of the WHO International Classification of Impairments, Activities and Participation (ICIDH-2) be adopted in the field of leprosy, particularly for the areas of prevention of impairment and disability and rehabilitation. The "WHO disability grade" should be renamed "WHO impairment grade."—Authors' Summary

Other Mycobacterial Diseases and Related Entities

Actor, J. K., Olsen, M., Jagannath, C. and Hunter, R. L. Relationship of survival, organism containment, and granuloma formation in acute marine tuberculosis. J. Interferon Cytokine Res. **19** (1999) 1183–1193.

The relationship among organism growth, immunopathology, and survival was studied in C57BL/6 and A/J mice acutely infected with *Mycobacterium tuberculosis* (MTB) (Erdman). Although organisms grew at similar rates in the lungs of both mouse strains, A/J mice died prior to 14 days after infection; whereas C57BL/6 mice survived twice as long. The lungs of A/J mice exhibited necrotizing interstitial inflammation and widely distributed acid-fast bacilli (AFB) without granuloma formation. In contrast, the lungs of C57BL/6 mice had relatively mild interstitial inflammation, which was replaced by focal granulomas, and AFB were primarily within granulomas. MTB induced similar granulomas for A/J and C57BL/6 mice in spleen and liver. In the lung, the A/J mice produced only transient messages for interferon-gamma (IFN-γ), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), IL-10, and inducible nitric oxide synthase (iNOS). The C57BL/6 mice, in contrast, produced a delayed but sustained response in the lung correlating with granuloma onset and characterized by high induction of IL-6, IFN-γ, IL-1β, IL-10, and TNF-α. Responses in the liver and spleen were also evaluated. These results demonstrate that histopathology and cytokine response to MTB infection varies among organs in mice. Increased survival during acute infection may, therefore, depend on the ability to contain organisms within granulomas in the lung.—Authors' Abstract

Agranoff, D., Monahan, I. M., Mangan, J. A., Butcher, P. D. and Krishna, S. *Mycobacterium tuberculosis* expresses a novel pH-dependent divalent cation transporter belonging to the Nramp family. J. Exp. Med. **190** (1999) 717–724.

Mammalian natural resistance-associated macrophage protein (Nramp) homologs are important determinants of susceptibility to infection by diverse intracellular pathogens including mycobacteria. Eukaryotic Nramp homologs transport divalent cations such as Fe2+, Mn2+, Zn2+, and Cu2+. *Mycobacterium tuberculosis* and *M. bovis* (bacillus Calmette-Guerin [BCG]) also encode an Nramp homolog (Mramp).

RNA encoding Mramp induces similar to 20-fold increases in Zn-65(2+) and Fe-55(2+) uptake when injected into *Xenopus laevis* oocytes. Transport is dependent on acidic extracellular pH and is maximal between pH 5.5 and 6.5. Mramp-mediated Zn-65(2+) and 55Fe(2+) transport is abolished by an excess of Mn2+ and Cu2+, confirming that Mramp interacts with a broad range of divalent transition metal cations.

Using semiquantitative reverse transcription PCR, we show that Mramp mRNA levels in *M. tuberculosis* are upregulated in response to increases in ambient Fe2+ and Cu2+ between <1 and 5 μM concentrations and that this upregulation occurs in parallel with mRNA for y39, a putative metal-transporting P-type ATPase. Using a quantitative ratiometric PCR technique, we demonstrate a fourfold decrease in Mramp/y39 mRNA ratios from organisms grown in 5–70 μM Cu2+. *M. bovis* BCG cultured axenically and within THP-1 cells also expresses mRNA encoding Mramp.

Mramp exemplifies a novel prokaryotic class of metal ion transporter. Within

phagosomes, Mramp and Nramp1 may compete for the same divalent cations, with implications for intracellular survival of mycobacteria.—Authors' Abstract

Bachhawat, N. and Mande, S. C. Identification of the INO1 gene of *Mycobacterium tuberculosis* H37Rv reveals a novel class of inositol-1-phosphate synthase enzyme. J. Mol. Biol. **291** (1999) 531–536.

1L-myo-inositol (inositol) is vital for the biogenesis of mycothiol, phosphatidylinositol and glycosylphophatidylinositol anchors linked to complex carbohydrates in *Mycobacterium tuberculosis.* All these cellular components are thought to play important roles in host-pathogen interactions and in the survival of the pathogen within the host. However, the inositol biosynthetic pathway in *M. tuberculosis* is not known. To delineate the pathways for inositol formation, we employed a unique combination of tertiary structure prediction and yeast-based functional assays. Here we describe the identification of the gene for mycobacterial INO1 that encodes inositol-1-phosphate synthase distinct in many respects from the eukaryotic analogs.—Authors' Abstract

Benini, J., Ehlers, E. M. and Ehlers, S. Different types of pulmonary granuloma necrosis in immunocompetent vs. TNFRp55-gene-deficient mice aerogenically infected with highly virulent *Mycobacterium avium.* J. Pathol. **189** (1999) 127–137.

The immunopathogenesis of mycobacterial infections frequently involves the formation of caseating granulomas which cause tissue destruction and, in the case of tuberculosis (TB), may lead to cavity formation. Both intravenous and aerosol models of *Mycobacterium tuberculosis* infection in mice do not reflect the pulmonary lesions characteristic of TB patients. Using both low-dose (10^2 colony-forming units, cfu) and high-dose (10^5 cfu) aerosol infection with a highly virulent strain of *M. avium* (TMC724) in C57BL/6 mice, it is now shown that these mice are capable of developing centrally caseating necrosis in lung granulomas after approximately 4 months of infection. In contrast, mice infected intravenously with the high-dose never developed this type of lesion, although bacterial counts in their lungs reached levels comparable to those attained by aerosol-infected mice (10^{10} cfu). To study the relevance of events signalled by tumor necrosis factor (TNF) in this model, TNFRp55 gene-deficient and syngeneic C57BL/6 immunocompetent mice were infected with 10^5 cfu *M. avium* via aerosol. In gene-deficient mice, newly formed pulmonary granulomas acutely disintegrated, showing sings of apoptotic cell death and neutrophil influx, and TNFRp55 knock-out mice all succumbed to infection just beyond the stage of granuloma initiation. Aerogenic infection with *M. avium* in mice is a suitable model to study the immunopathogenesis of granuloma necrosis because it closely mimics the histopathology of mycobacterial infections in humans, including TB. Furthermore, the use of TNFRp55 gene-deficient mice in this model establishes a role for TNF in maintaining the integrity of a developing pulmonary granuloma.—Authors' Abstract

Bermudez, L. E., Kolonoski, P., Wu, M., Aralar, P. A., Inderlied, C. B. and Young, L. S. Mefloquine is active *in vitro* and *in vivo* against *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **43** (1999) 1870–1874.

Despite the development of several agents, new classes of antimicrobials with activity against the *Mycobacterium avium* complex (MAC) are needed. Based on a broad screening of compounds, we found that mefloquine has MICs of 8 to 16 μg/ml by the BACTEC system and 16 μg/ml by broth microdilution for five MAC strains tested. An expansion of the screening with broth microdilution to 24 macrolide-susceptible strains and 6 macrolide-resistant strains determined that the MIC for all strains was 16 μg/ml. To determine the intracellular activity of mefloquine, U937 macrophage monolayers infected with MAC strain 101, 100, or 109 (serovars 1, 8, and 4) were treated with mefloquine daily, and the number of intracellular bacteria was quantitated after 4 days. Significant growth inhibition against the three MAC strains at

concentrations greater than or equal to 10 μg/ml ($p < 0.05$) was obtained. Due to the encouraging anti-MAC activity, *in vivo* efficacy in beige mice infected with MAC 101 was evaluated. Animals were treated with 5, 10, 20, or 40 mg/kg of body weight daily, three times a week, twice a week, or once a week for 4 weeks, and bacteria were quantitated in blood, liver, and spleen. No toxicity was observed with any of the treatment regimens. Mefloquine had borderline bactericidal activity at a dosage of 40 mg/kg daily (100% inhibition compared with a 1-week control), and significant inhibition was obtained at dosages of 40 mg/kg three times a week, as well as 20 mg/kg daily. Mefloquine had no significant effect on bacteremia. A combination of mefloquine and ethambutol showed significantly more activity than did either drug alone in liver, spleen, and blood; the combination was also bactericidal against *M. avium*. Although safety is a potential concern, mefloquine and related compounds deserve further investigation as anti-MAC therapies.—Authors' Abstract

Bermudez, L. E., Wu, M., Miltner, E. and Inderlied, C. B. Isolation of two subpopulations of *Mycobacterium avium* within human macrophages. FEMS Microbiol. Lett. **178** (1999) 19–26.

Mycobacterium avium is an intracellular pathogen that is associated with disseminated infection in acquired immunodeficiency syndrome (AIDS) patients. Human monocyte-derived macrophages were infected with *M. avium* strain 101 and a quinolone (Bay y 3118) was used at 8 μg ml^{-1}, a concentration that kills growing bacteria but fails to eliminate static organisms. Infected monolayers were treated with Bay y 3118 for 4 days and viable bacteria obtained from the lysis of macrophages were used to infect other macrophages without passage in media. The procedure was repeated five times, after which seven different subpopulations that failed to grow within macrophages were identified. While the DNA fingerprinting confirmed that all came from the same strain, three protein profiles were observed. Static subpopulations were not killed by cytokine-stimulated macrophages, in contrast to the replicating subpopulation. Three of the static subpopulation trains were shown to be auxotrophic for glutamic acid or methionine. All seven nonduplicating subpopulation strains grew well in complete 7H10 agar. The importance of a static subpopulation of *M. avium* within macrophages is presently unknown. It is possible, however, that the nongrowing bacteria would persist within macrophages.—Authors' Abstract

Billington, O. J., McHugh, T. D. and Gillespie, S. H. Physiological cost of rifampin resistance induced *in vitro* in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **43** (1999) 1866–1869.

Drug-resistant *Mycobacterium tuberculosis* is a major threat to public health. In clinical practice, a limited number of resistance mutations in a short sequence of the beta subunit of RNA polymerase (encoded by rpoB) have been described. Spontaneous resistance to rifampin was induced *in vitro* in *M. tuberculosis* H37Rv (ATCC 9360). Only three resistance patterns could be detected by PCR-single-strand conformation polymorphism analysis. Sequence analysis revealed that Ser(531) → Leu arose most frequently, followed by His(526) → Arg and then either His(526) → Tyr or His(526) → ASP. The relative Darwinian fitness of all but one of the mutant genotypes was less than that of the susceptible parent and, for these mutations, there was a significant correlation between fitness and clinical isolation rate (regression analysis $p = 0.026$). The fitness deficit in some mutants was small, suggesting that there is little likelihood of a spontaneous reversion to susceptibility.—Authors' Abstract

Couture, M., Yeh, S. R., Wittenberg, B. A., Wittenberg, J. B., Ouellet, Y., Rousseau, D. L. and Guertin, M. A cooperative oxygen-binding hemoglobin from *Mycobacterium tuberculosis*. Proc. Natl. Acad. Sci. U.S.A. **96** (1999) 11223–11228.

Two putative hemoglobin genes, glbN and glbO, were recently discovered in the complete genome sequence of *Mycobacterium tuberculosis* H37Rv. Here we show

that the glbN gene encodes a dimeric hemoglobin (HbN) that binds oxygen cooperatively with very high affinity (P-50 = 0.013 mmHg at 20°C) because of a fast combination (25 $\mu M^{-1} \cdot s^{-1}$) and a slow dissociation (0.2 s^{-1}) rate. Resonance Raman spectroscopy and ligand association/dissociation kinetic measurements, along with mutagenesis studies, reveal that the stabilization of the bound oxygen is achieved through tyrosine at the B10 position in the distal pocket of the heme with a conformation that is unique among the globins. Physiological studies performed with *M. bovis* bacillus Calmette-Guerin demonstrate that the expression of HbN is greatly enhanced during the stationary phase in aerobic cultures but not under conditions of limited oxygen availability. The results suggest that, physiologically, the primary role of HbN may be to protect the bacilli against reactive nitrogen species produced by the host macrophage.—Authors' Abstract

Duong, D. J., Spigel, G. T., Moxley, R. T., III, and Gaspari, A. A. American experience with low-dose thalidomide therapy for severe cutaneous lupus erythematosus. Arch. Dermatol. **135** (1999) 1079–1087.

Background: There is a renewed interest in thalidomide therapy after its surprising effectiveness in treating erythema nodosum leprosum was first published. Thalidomide has subsequently been reported to be effective in treating a number of dermatoses, including cutaneous lupus erythematosus. We examined the efficacy and adverse effects of low-dose, long-term thalidomide monotherapy in 7 patients with various forms of cutaneous lupus erythematosus that were unresponsive to traditional systemic treatments.

Observations: Six of the 7 patients treated with thalidomide after discontinuation of other oral agents had complete or marked resolution of their previously treatment-resistant cutaneous lesions, with an average response time of 2.2 ± 0.8 months. Our cohort of 7 patients with cutaneous lupus erythematosus was treated with thalidomide therapy for an average of 2.4 ± 3.1 years (range, 1 month to 9 years). The most common adverse effects were sedation, constipation, and weight gain. Two patients reported experiencing intermittent shaking episodes, an adverse effect not previously reported in the literature. Four patients reported symptoms of paresthesia, but none was found to be caused by thalidomide-induced peripheral neuropathy.

Conclusions: A low starting dose of thalidomide as a monotherapy with continued sun avoidance is a safe and effective treatment for the various cutaneous manifestations of lupus erythematosus after traditional therapeutic options have failed to control disease. Our experience with low-dose, long-term thalidomide therapy suggests that peripheral neuropathy is not as common as suggested by other studies (up to 50% of patients treated with thalidomide in some series).—Authors' Abstract

Falkson, C. I. and Falkson, G. A phase II evaluation of clofazimine plus doxorubicin in advanced, unresectable primary hepatocellular carcinoma. Oncology **57** (1999) 232–235.

The riminophenazine compound clofazimine has been shown to be a potent inhibitor of hepatocellular carcinoma (HCC) *in vitro*. Therapeutic benefit was claimed for patients with HCC treated with clofazimine in a recent clinical trial. The current trail was initiated to evaluate response and survival of patients with HCC receiving clofazimine plus doxorubicin. Twenty-eight patients were entered into the study, of whom 27 were evaluable for response and survival. No patients had a complete or partial response, and 9 had stable disease. The median survival time was 7 weeks. Toxicity was mild with yellow pigmentation of the skin resulting from the clofazimine, and leukopenia, nausea, vomiting and mucositis as expected from doxorubicin. Further studies using other riminophenazine compounds are warranted.—Authors' Abstract

Gilbertson, B., Zhong, J. and Cheers, C. Anergy, IFN-gamma production, and apoptosis in terminal infection of mice with *Mycobacterium avium*. J. Immunol. **163** (1999) 2073–2080.

We have followed the course of experimental infection of mice with *Mycobac-*

terium avium over an extended period, assessing bacterial numbers and T-cell responsiveness. When mice were infected intranasally, bacteria spread to the spleen and liver, but remained in highest numbers in the lungs. Both CD4+ and CD8+ T cells, assayed at any time from 6–28 wk after infection, produced gamma interferon (IFN-γ). After initial rapid growth, bacterial numbers slowly increased from similar to 10^7 at 6 wks to more than 5×10^8 at 28 wks, indicating that the resistance mechanisms so generated were not adequate to contain the infection. During infection, apoptosis of both CD4+ and CD8+ T cells, measured immediately *ex vivo* by staining with Annexin V, increased steadily. With some individual exceptions, there was a close correlation between apoptosis of CD4+ cells and level of IFN-γ production by cultured spleen cells. By 34 wks postinfection, there was an abrupt cessation of IFN-γ production. No IL-4 was detected, ruling out a switch to Th2 profile. Subsequently, bacterial numbers increased still further to $>5 \times 10^9$ per lung, and the mice lost body weight and would have died if not killed for experimental or humane reasons. The possibility that T cells exposed over this prolonged period to extremely high doses of Ag may become tolerant by a process of terminal differentiation is discussed.—Authors' Abstract

Graham, J. E. and Clark-Curtiss, J. E. Identification of *Mycobacterium tuberculosis* RNAs synthesized in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS). Proc. Natl. Acad. Sci. U.S.A. **96** (1999) 11554–11559.

A widely applicable, positive cDNA selection method was developed to identify RNAs synthesized by *Mycobacterium tuberculosis* in response to phagocytosis by cultured human primary macrophages. cDNAs for sigE and sigH (alternative sigma factors), aceA (isocitrate lyase), ponA (class I penicillin-binding protein), pks2 (polyketide synthase), uvrA (UvrABC endonuclease), and ctpV (putative cation transporter) were obtained from macrophage-grown bacteria. cDNAs for ORFs Rv3070, Rv3483c, Rv0903c (encoding a putative bacterial two-component transcriptional activator), and Rv0170 of the mce1 virulence operon also were obtained from phagocytized bacilli. cDNAs for these genomic regions were not obtained form approximately 1000-fold more bacteria grown in laboratory broth. Methods described here, which have identified *M. tuberculosis* genes expressed in response to host interaction, will allow the study of gene expression in a variety of microorganisms, including expression resulting from interaction with human tissues in natural disease states.—Authors' Abstract

Haslett, P. A. J., Klausner, J. D., Makonkawkeyoon, S., Moreira, A., Metatratip, P., Boyle, B., Kunachiwa, W., Maneekarn, N., Vongchan, P., Corral, L. G., Elbeik, T., Shen, Z. and Kaplan, G. Thalidomide stimulates T cell responses and interleukin 12 production in HIV-infected patients. AIDS Res. Hum. Retroviruses **15** (1999) 1169–1179.

We performed a placebo-controlled study to evaluate the effects of immunomodulatory treatment with thalidomide on HIV levels, TNF-alpha levels, and immune status of 31 HIV-infected individuals, after temporary suppression of viral replication with antiretroviral drugs. Treatment with a combination of zidovudine and lamivudine (ZDV/LMV) for 14 days resulted in a median decline in plasma viremia of 1.93 $\log^{10}$, RNA equivalents/ml. After discontinuation of ZDV/LMV, thalidomide therapy (200 mg/day for 4 weeks) did not retard the prompt return of HIV titers to the pretreatment levels, and had no effect on plasma levels of TNF-alpha. In contrast, thalidomide treatment resulted in significant immune stimulation. We observed increased levels of plasma soluble IL-2 receptor, soluble CD8 antigen, and IL-12 ($p < 0.01$ for all parameters), as well as increased cutaneous delayed-type hypersensitivity reactions to recall antigens ($p < 0.01$) in thalidomide-treated patients. These changes were associated with a median increase in HIV titer of 0.2 $\log^{10}$ RNA equivalents/ml in the thalidomide-treated group ($p < 0.05$), which resolved after stopping the drug.

Further studies were performed *in vitro* to elucidate the mechanism of thalidomide-induced immune stimulation. When purified T cells from HIV-infected individuals were stimulated by immobilized anti-CD3 in the presence of thalidomide, a costimulatory effect of the drug was observed, resulting in increased production of IL-2 and IFN-gamma, and increased T-cell proliferative responses. Further experiments showed that thalidomide increased IL-12 production by antigen-presenting cells in a T-cell-dependent manner. Our findings suggest a potential application for thalidomide as a novel immune adjuvant in HIV disease.—Authors' Abstract

Hussain, S., Zwilling, B. S. and Lafuse, W. P. *Mycobacterium avium* infection of mouse macrophages inhibits IFN-gamma Janus kinase-STAT signaling and gene induction by downregulation of the IFN-gamma receptor. J. Immunol. **163** (1999) 2041–2048.

Macrophage activation is required to control the growth of intracellular pathogens. Recent data indicate that macrophages become functionally deactivated during mycobacterial infection. We studied macrophage deactivation by examining the expression of a panel of IFN-gamma-inducible genes and activation of Janus Kinase (JAK)-STAT pathway in *Mycobacterium avium*-infected macrophages. Reduced expression of IFN-gamma-inducible genes-MHC class II gene EP; MHC class II transactivator; IFN regulatory factor-1; and Mg21, a gene coding for a GTP-binding protein, was observed in *M. avium*-infected macrophages. Decreased tyrosine phosphorylation and DNA binding activity of STAT1 in *M. avium*-infected macrophages stimulated with IFN-gamma was observed. Tyrosine phosphorylation of JAK1, JAK2, and IFN-gamma R alpha was also reduced in infected cells. Northern and Western blot analyses showed that a downregulation of IFN-gamma R alpha- and beta-chain mRNA and protein occurred in *M. avium*-infected macrophages. The downregulation of IFN-gamma R and inhibition of STAT1 activation were time dependent and required 4 hr of infection for downregulation of the IFN-gamma R and 8 hr for STAT1 inhibition. These findings suggest that *M. avium* infection inhibits induction of IFN-gamma-inducible genes in mouse macrophages by downregulating IFN-gamma R, resulting in reduced phosphorylation of IFN-gamma R alpha, JAK1, JAK2, and STAT1.—Authors' Abstract

Kartmann, B., Stengler, S. and Niederweis, M. Porins in the cell wall of *Mycobacterium tuberculosis.* J. Bacteriol. **181** (1999) 6543–6546.

Lipid bilayer experiments indicated that the cell wall of *Mycobacterium tuberculosis* contains at least two different porins: (i) a cation-selective, heat-sensitive 0.7-nS channel which has a short-lived open state and is probably composed of 15-kDa subunits and (ii) a 3-nS, >60-kDa channel with a long-lived open state, resembling porins from fast-growing mycobacteria.—Authors' Abstract

Keravich, D. P. and Daniels, C. E. Challenges of thalidomide distribution in a hospital setting. Am. J. Health-System Pharm. **56** (1999) 1721–1725.

The various physician, patient, and pharmacy requirements for participation in the System for Thalidomide Education and Prescribing Safety (STEPS) program and procedures that institutions may implement in order to comply with these requirements are described.

In 1998, FDA approved the marketing of thalidomide (Thalomid, Celgene). Because of the drug's known teratogenic effects, FDA tightly controls the distribution of thalidomide in the United States. To comply with FDA requirements, Celgene developed the STEPS Oversight program, which includes registration of thalidomide prescribers and pharmacies that dispense thalidomide, extensive patient eduction about the risks associated with thalidomide, and a registry of all patients receiving thalidomide. The STEPS Program is considered part of the product label. The pharmacy requirements of the program were developed with a focus on a retail pharmacy practice model, which does not adequately reflect current hospital practice. The pharmacy department of the National Institutes

of Health Clinical Center developed a model that adapts the STEPS Program requirements to inpatient and outpatient institutional pharmacy practice.

Procedures for registering patients and prescribers and dispensing thalidomide in the hospital setting were developed; the procedures were designed to meet the needs of both the inpatient and outpatient pharmacies and to comply with the requirements of the STEPS Program.—Authors' Abstract

Kim, K. D., Lee, H. G., Kim, J. K., Park, S. N., Choe, I. S., Choe, Y. K., Kim, S. J., Lee, E. and Lim, J. S. Enhanced antigen-presenting activity and tumour necrosis factor-alpha-independent activation of dendritic cells following treatment with *Mycobacterium bovis* bacillus Calmette-Guerin. Immunology **97** (1999) 626–633.

Dendritic cells (DCs) are most potent among the antigen-presenting cells and are believed to be crucial for the initiation of a primary T-cell response to foreign antigens. Mycobacterial infection within macrophages is controlled by cell-mediated immunity. To elucidate the stimulation of immune response by *Mycobacterium bovis* bacillus Calmette-Guerin (BCG), we purified DCs from precursor cells in human peripheral blood mononuclear cells (PBMC) by culturing them with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) and characterized their surface antigen expression. The interaction of cultured DCs with BCG resulted in increased surface expression of several DC-related marker antigens. BCG also induced reduction of endocytosis, enhancement of CD83 expression as well as B7 costimulatory molecules and IL-12 production, suggesting that BCG treatment directly induces DCs to mature. BCG-treated DCs were much more potent antigen-presenting cells in allogeneic immune response than untreated DCs. Moreover, while the neutralization of tumor necrosis factor-alpha (TNF-α) significantly blocked the DC maturation induced by lipopolysaccharide (LPS), it could not inhibit the induction of DC maturation by the BCG treatment, indicating that TNF-α production plays a minor role in the BCC-induced DC maturation. However, the neutralization of TNF-α resulted in decreased IL-12 production by activated DCs. These results suggest that infection with BCG might evoke direct activation and maturation of DC and the general immune stimulant effect of BCG might be related with the activation of DCs.—Authors' Abstract

Lall, N. and Meyer, J. J. M. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. J. Ethnopharmacol. **66** (1999) 347–354.

Twenty South African medicinal plants used to treat pulmonary diseases were screened for activity against drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis.* A preliminary screening of acetone and water plant extracts against a drug-sensitive strain of *M. tuberculosis* H37Rv was done by the agar plate method. Fourteen of the 20 acetone extracts showed inhibitory activity at a concentration of 0.5 mg/ml against this strain. Acetone as well as water extracts of *Cryptocarya latifolia, Euclea natalensis, Helichrysum melanacme, Nidorella anomala* and *Thymus vulgaris* inhibited the growth of *M. tuberculosis.* Given the activity of 14 acetone extracts at 0.5 mg/ml against the drug-sensitive strain by the agar plate method, a further study was done employing a rapid radiometric method to confirm the inhibitory activity. These active acetone extracts were screened against the H37Rv strain as well as a strain resistant to the drugs isoniazid and rifampin. The minimal inhibitory concentration of *Croton pseudopulchellus, Ekebergia capensis, Euclea natalensis, Nidorella anomala* and *Polygala myrtifolia* was 0.1 mg/ml against the H37Rv strain by the radiometric method. Extracts of *Chenopodium ambrosioides, Ekebergia capensis, Euclea natalensis, Helichrysum melanacme, Nidorella anomala* and *Polygala myrtifolia* were active against the resistant strain at 0.1 mg/ml. Eight plants showed activity against both strains at a concentration of 1.0 mg/ml.—Authors' Abstract

Li, Z. M., Howard, A., Kelley, C., Delogu, G., Collins, F. and Morris, S. Immunogenicity of DNA vaccines expressing tuberculosis proteins fused to tissue plasminogen activator signal sequences. Infect. Immun. **67** (1999) 4780–4786.

Novel tuberculosis DNA vaccines encoding native ESAT-6, MPT-64, KatG, or HBHA mycobacterial proteins or the same proteins fused to tissue plasminogen activator (TPA) signal sequences were evaluated for their capacity to elicit humoral, cell-mediated, and protective immune responses in vaccinated mice. While all eight plasmids induced specific humoral responses, the constructs expressing the TPA fusions generally evoked higher antibody responses in vaccinated hosts. Although most of the DNA vaccines tested induced a substantial gamma interferon response in the spleen, the antigen-specific lung responses were 2- to 10-fold lower than the splenic responses at the time of challenge. DNA vaccines encoding the ESAT-6, MPT-64, and KatG antigens fused to TPA signal sequences evoked significant protective responses in mice aerogenically challenged with low doses of *Mycobacterium tuberculosis* Erdman 17 to 21 days after the final immunization. However, the protective response induced by live *M. bovis* BCG vaccine was greater than the response induced by any of the DNA vaccines tested. These results suggest that the tuberculosis DNA vaccines were able to elicit substantial immune responses in suitably vaccinated mice, but further refinements to the constructs or the use of alternative immunization strategies will be needed to improve the efficacy of these vaccine candidates.—Authors' Abstract

Means, T. K., Wang, S. Y., Lien, E., Yoshimura, A., Golenbock, D. T. and Fenton, M. J. Human Toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. J. Immunol. **163** (1999) 3920–3927.

Recent studies have implicated a family of mammalian Toll-like receptors (TLR) in the activation of macrophages by gram-negative and gram-positive bacterial products. We have previously shown that different TLR proteins mediate cellular activation by the distinct CD14 ligands gram-negative bacterial LPS and mycobacterial glycolipid lipoarabinomannan (LAM). Here we show that viable *Mycobacterium tuberculosis* bacilli activated both Chinese hamster ovary cells and murine macrophages that overexpressed either TLR2 or TLR4. This contrasted with gram-positive bacteria and *M. avium,* which activated cells via TLR2 but not TLR4. Both virulent and attenuated strains of *M. tuberculosis* could activate the cells in a TLR-dependent manner. Neither membrane-bound nor soluble CD14 was required for bacilli to activate cells in a TLR-dependent manner. We also assessed whether LAM was the mycobacterial cell wall component responsible for TLR-dependent cellular activation by *M. tuberculosis.* We found that TLR2, but not TLR4, could confer responsiveness to LAM isolated from rapidly growing mycobacteria. In contrast, LAM isolated from *M. tuberculosis* or *M. bovis* bacillus Calmette-Guerin failed to induce TLR-dependent activation. Lastly, both soluble and cell wall-associated mycobacterial factors were capable of mediating activation via distinct TLR proteins. A soluble heat-stable and protease-resistant factor was found to mediate TLR2-dependent activation; whereas a heat-sensitive cell-associated mycobacterial factor mediated TLR4-dependent activation. Together, our data demonstrate that Toll-like receptors can mediate cellular activation by *M. tuberculosis* via CD14-independent ligands that are distinct from the mycobacterial cell wall glycolipid LAM.—Authors' Abstract

Morrison, A., Gyure, K. A., Stone, J., Wong, K., McEvoy, P., Koeller, K. and Mena, H. Mycobacterial spindle cell pseudotumor of the brain—a case report and review of the literature. Am. J. Surg. Pathol. **23** (1999) 1294–1299.

Spindle cell pseudotumors found in the skin, lymph nodes, bone marrow, spleen, lungs, and retroperitoneum have been reported recently in immunosuppressed patients, including those with acquired immunodeficiency syndrome. The authors report a similar lesion limited to the brain in a

38-year-old human immunodeficiency virus-negative man receiving steroid therapy for treatment of sarcoidosis. Histopathologically, the lesions were composed of spindle and epithelioid histiocytes, small foci of necrosis, and numerous acid-fast bacilli (AFB). The AFB were determined by culture and polymerase chain reaction to be *Mycobacterium avium-intracellulare.* Because of the uncommon histologic appearance of this lesion and the potential for treatment if recognized, mycobacterial spindle cell pseudotumors should be included in the differential diagnosis of spindle cell lesions in the brain in immunosuppressed patients.—Authors' Abstract

Pahlevan, A. A., Wright, D. J. M., Andrews, C., George, K. M., Small, P. L. C. and Foxwell, B. M. The inhibitory action of *Mycobacterium ulcerans* soluble factor on monocyte/T cell cytokine production and NF-kappa B function. J. Immunol. **163** (1999) 3928–3935.

Buruli ulcer is a chronic and progressive necrotizing ulcer for which there is no medical treatment. Historically, a soluble toxin (factor) derived from the causative *Mycobacterium ulcerans* was found to induce the massive necrosis of skin and s.c. tissue seen in this condition. However, the persistence of the disease is thought to be caused by a lack of any immune response. We therefore investigated whether the factor was related to immunosuppression. A protocol to partially purify the factor was developed, and its effects on immune competent cells were tested. The factor produced >95% inhibition of LPS-induced release of TNF and IL-10 from human monocytes and caused a loss of adherence of these cells without cell death. The factor also blocked the production of IL-2 from activated T lymphocytes. The factor had no effect on TNF-induced cytotoxicity, but abrogated TNF-induced NF-kappa B activation. Surprisingly, a synergy was observed between the factor and phorbol ester directed NF-kappa B activation. The factor had no effect on IL-1- or LPS-induced NF-kappa B activity, indicating selective activity of the factor. The factor did not inhibit the degradation of I kappa B alpha induced by TNF, indicating that the target for its activity lies within an undefined part of the TNF signaling mechanism. The data indicate that the localized immunosuppression associated with Buruli ulcer relates to the activity of the released factor, and this may provide a target for future therapeutic strategies for this intractable disease.—Authors' Abstract

Perera, J. and Arachchi, D. M. The optimum relative centrifugal force and centrifugation time for improved sensitivity of smear and culture for detection of *Mycobacterium tuberculosis* from sputum. Trans. R. Soc. Trop. Med. Hyg. **93** (1999) 405–409.

Direct microscopy is the only available method for diagnosis of tuberculosis in most centers in developing countries. Methods to improve the sensitivity of direct smear are an urgent requirement. Sputum specimens artificially seeded with known concentrations of *Mycobacterium tuberculosis* were liquefied and decontaminated with sodium hydroxide-sodium citrate-N-acetyl-L-cysteine solutions. They were subjected to different centrifugation forces and centrifugation times after which the centrifuged deposits were examined by smear and culture. Statistical analysis of results was carried out using EpiInfo version 6.0. The optimum relative centrifugal force (RCF) and centrifugation time combination was 4000 g for 15 min. The sensitivity of detection at an RCF of 4000 g for 15 min was 5000 organisms/mL and 500 organisms/mL for smear and culture, respectively. When results of 163 clinical samples were analyzed after centrifugation at 4000 g for 15 min sensitivity of the direct smear improved from 63% to 92% ($p < 0.05$) and negative predictive value from 30.5% to 45% ($p < 0.05$) when culture was considered the "gold standard." With the concentrated smear there was a reduction in specificity from 82% to 60% ($p > 0.05$). Because most laboratories are equipped with a simple centrifuge, smear sensitivity can be improved with this simple modification. The other advantage is that the same centrifuged deposit can be cultured, in contrast to when sodium hypochlorite is used for liquefaction.—Authors' Abstract

Roach, D. R., Briscoe, H., Baumgart, K., Rathjen, D. A. and Britton, W. J. Tumor necrosis factor (TNF) and a TNF-mimetic peptide modulate the granulomatous response to *Mycobacterium bovis* BCG infection *in vivo*. Infect. Immun. **67** (1999) 5473–5476.

Tumor necrosis factor (TNF) is a critical mediator in the immune response to mycobacteria, particularly in the formation and maintenance of granulomas. Treatment of *Mycobacterium bovis* BCG-infected mice with TNF and a TNF-mimetic peptide (TNF70–80) altered the number and cellular composition of granulomas. This change was associated with a moderate decrease in the bacterial burden.—Authors' Abstract

Sastry, P. S. R. K. Inhibition of TNF-alpha synthesis with thalidomide for prevention of acute exacerbations and altering the natural history of multiple sclerosis. Med. Hypotheses **53** (1999) 76–77.

Multiple sclerosis (MS) is a common neurological disorder which has a relapsing/remitting course and is presently incurable. A variety of agents have been tried to prevent exacerbations and alter the natural history of the disease. Tumor necrosis factor-alpha (TNF-α) has been implicated as the most important cytokine in the pathogenesis of MS. There is evidence that the thalidomide is an agent which blocks production of TNF-α by a mechanism different from other agents. Hence, it is hypothesized that using thalidomide as therapy would prevent acute exacerbations of MS as well as alter its natural history.—Author's Abstract

Teo, S. K., Colburn, W. A. and Thomas, S. D. Single-dose oral pharmacokinetics of three formulations of thalidomide in healthy male volunteers. J. Clin. Pharmacol. **39** (1999) 1162–1168.

Thalidomide was recently approved in the United States for the treatment of erythema nodosum leprosum, a complication of leprosy. The present study determined the bioequivalence and pharmacokinetics of Celgene's commercial and clinical trial thalidomide formulations and the Brazilian Tortuga formulation in an open-label, single-dose, three-way crossover design. Seventeen healthy subjects were given 200 mg of thalidomide on three occasions, and blood samples were collected over 48 hours. Pharmacokinetic parameters were determined using compartmental methods for the two Celgene formulations and using noncompartmental methods for all three formulations. All subjects reported adverse events, none of which was serious or unexpected. Celgene formulations were bioequivalent when comparing C-max t(max), and AUC. There was significant variability in plasma levels form the Tortuga formulation, giving a mean profile that was distinctly different from the two Celgene formulations with a lower C-max value and a longer terminal phase. The lower C-max was probably due to slower absorption. The terminal rate constant for the Tortuga formulation was significantly less, giving rise to a terminal half-life of 15 hr compared to about 5 to 6 hr for the Celgene formulations. Confidence intervals for C-max between the Tortuga and the Celgene formulations were outside the 80% to 125% range, indicating a lack of bioequivalence. Extent of absorption, as measured by AUC ($0 \leftarrow \alpha$) was approximately equal for all three formulations. Terminal half-life for Tortuga was two to three times longer compared to the Celgene formulations and is clear evidence for absorption rate limitations. The two Celgene formulations showed similar pharmacokinetic parameters with profiles that were best described by a one compartment model with first-order absorption and elimination. The authors conclude that Celgene's clinical trial and commercial thalidomide formulations are similar to each other and distinctly different from the Tortuga formulation and that all three formulations exhibited absorption rate-limited elimination.—Authors' Abstract

van der Werf, T. S., van der Graaf, W. T. A., Tappero, J. W. and Asiedu, K. *Mycobacterium ulcerans* infection. Lancet **354** (1999) 1013–1018.

After tuberculosis and leprosy, Buruli-ulcer disease (caused by infection with *Mycobacterium ulcerans*) is the third most common mycobacterial disease in immuno-

competent people. Countries in which the disease is endemic have been identified, predominantly in areas of tropical rain forest; the emergence of Buruli-ulcer disease in West African countries over the past decade has been dramatic. Current evidence suggests that the infection is transmitted through abraded skin or mild traumatic injuries after contact with contaminated water, soil, or vegetation; there is one unconfirmed preliminary report on possible transmission by insects. The clinical picture ranges from a painless nodule to large, undermined ulcerative lesions that heal spontaneously but slowly. Most patients are children. The disease is accompanied by remarkably few systemic symptoms, but occasionally secondary infections resulting in sepsis or tetanus cause severe systemic disease and death. Extensive scarring can lead to contractures of the limbs, blindness, and other adverse sequelae, which impose a substantial health and economic burden. Treatment is still primarily surgical, and includes excision, skin grafting, or both. Although BCG has a mild but significant protective effect, new vaccine developments directed at the toxins produced by *M. ulcerans* are warranted. In West Africa, affected populations are underprivileged, and the economic burden imposed by Buruli-ulcer disease is daunting. Combined efforts to improve treatment, prevention, control, and research strategies (overseen by the WHO and funded by international relief agencies) are urgently needed.—Authors' Abstract

Van Rie, A., Warren, R., Richardson, M., Victor, T. C., Gie, R. P., Enarson, D. A., Beyers, N. and van Helden, P. D. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. N. Engl. J. Med. **341** (1999) 1174–1179.

Background: For decades it has been assumed that postprimary tuberculosis is usually caused by reactivation of endogenous infection rather than by a new, exogenous infection.

Methods: We performed DNA fingerprinting with restriction-fragment-length polymorphism analysis on pairs of isolates of *Mycobacterium tuberculosis* from 16 compliant patients who had a relapse of pulmonary tuberculosis after curative treatment of postprimary tuberculosis. The patients lived in areas of South Africa where tuberculosis is endemic. Medical records were reviewed for clinical data.

Results: For 12 of the 16 patients, the restriction-fragment-length polymorphism banding patterns for the isolates obtained after the relapse were different from those for the isolates from the initial tuberculous disease. This finding indicates that reinfection was the cause of the recurrence of tuberculosis after curative treatment. Two patients had reinfections with a multidrug-resistant strain. All 15 patients who were tested for the human immunodeficiency virus were seronegative.

Conclusions: Exogenous reinfection appears to be a major cause of postprimary tuberculosis after a previous cure in an area with a high incidence of this disease. This finding emphasizes the importance of achieving cures and of preventing anyone with infectious tuberculosis from exposing others to the disease.—Authors' Abstract

Zumarraga, M. J., Bernardelli, A., Bastida, R., Quse, V., Loureiro, J., Cataldi, A., Bigi, F., Alito, A., Castro Ramos, M., Samper, S., Otal, I., Martin, C. and Romano, M. I. Molecular characterization of mycobacteria isolated from seals. Microbiology **145** (1999) 2519–2526.

Tuberculosis (TB) was diagnosed in 10 seals from three species (*Arctocephalus australis, Arctocephalus tropicalis* and *Otaria flavescens*) found in South America. The mycobacteria isolated from these cases belonged to the *Mycobacterium tuberculosis* complex, as determined by RFLP using an IS*6110* probe, spoligotyping, analysis of the 16S rRNA gene sequence and by PCR-restriction analysis of *hsp*65. Polymorphisms in *gyrA, katG, oxyR* and *pncA* were investigated in some of the isolates, as well as the presence of the MPB70 antigen. The insertion sequence IS*6110* was present in 3 to 7 copies in the genome of the mycobacteria isolated from seals. Using the IS*6110* probe, 6 patterns (designated A, B, C, D, E and F) were identified from 10 different isolates. Patterns A and B were found for the mycobacteria isolated from 2 and 4 seals,

respectively, indicating an epidemiological relationship between isolates grouped according to their IS*6110* RFLP. The mycobacteria isolated from seals shared the majority of their IS*6110* DNA-containing restriction fragments, and 9 isolates had an identical spoligotype; only 1 isolate showed a minor difference in its spoligotype. In addition, none of these spoligotypes were found in other *M. tuberculosis* complex strains. These results suggest that the isolates from seals constitute a unique group of closely related strains. The mycobacteria isolated from seals showed polymorphisms at *gyrA* codon 95 and *katG* codon 463, as do group 1 *M. tuberculosis* and *M. bovis*. Group 1 mycobacteria are associated with cluster cases. The spoligotypes found in the mycobacteria isolated from seals lack spacers 39–43, as does *M. bovis,* but the MPB70 antigen, which is highly expressed in *M. bovis* and minimally expressed in *M. tuberculosis,* was not detected in these mycobacteria. The mycobacteria isolated from seals also showed *oxyR* and *pncA* polymorphisms specific to *M. tuberculosis.* In conclusion, the mycobacteria that cause TB in seals in the South-Western Atlantic are a related group, and based on the combination of genetic characteristics, belong to a unique genotypic group within the *M. tuberculosis* complex.—Authors' Abstract