CURRENT LITERATURE

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

General and Historical

Green, A. T. and Jochem, K. Sustaining leprosy services in the changing context of health sector reform. Lepr. Rev. **69** (1998) 134–144.

National leprosy control programs currently face a number of changes to the environment within which they operate. This paper examines the issues arising from these. It focuses, in particular, on those arising from changes in the structure of the health sector as a result of policies of health sector reform which are being considered or adopted in many developing countries. These include decentralization, financing strategies, greater role for the private and NGO sectors and the integration of vertical programs. The paper is structured around a number of key steps in the development of a strategy for sustainability of appropriate leprosy services. These are the assessment of the epidemiological, social and health services context, development of program objectives, planning of human and financial resources, development of the strategy, mapping the roles of potential actors, development of regulatory and incentive mechanism, action planning and managing change and, finally, re-evaluation of the program objectives and service delivery organization. The paper stresses the importance of process in developing ownership of a strategy. It concludes with a set of key questions which it suggests need to be addressed by leprosy program managers in the development of a pro-active response to the changes. —Authors' Summary

Lever, P., Bijlmaker, L., Zwanikken, P. and Saunderson, P. Health Systems Research in leprosy control—what contributions can it make? Lepr. Rev. **69** (1998) 122–127.

The paper describes a Health Systems Research (HSR) training program which took place at the All-Africa Leprosy, Tuberculosis and Rehabilitation Training Centre (ALERT) in Ethiopia. The training consisted of three stages: an initial workshop focussing on protocol development, followed by a fieldwork period and a data analysis and report writing workshop. Twenty participants, divided over four groups, took part in the training and carried out the research alongside their day-to-day professional commitments. Three of the projects were concerned with prevention of disabilities, one with integration of the leprosy program into the general health services. Based on the findings of their research, each group produced a set of recommendations and a plan of action for the implementation of these recommendations. The contribution of HSR to leprosy control is discussed.—Authors' Summary

Malin, A. and McAdam, K. P. W. J., eds. *Mycobacterial Diseases Part I: Clinical Frontiers.* Bailliere's Clin. Infect. Dis. **4** (1997) xvi + 118 pp.

This book is one of a set of two which cover all mycobacterial diseases to allow consideration of the similarities between various mycobacterial diseases as well as the differences; 13 authors have contributed to five chapters. The topics discussed include: management of leprosy reactions; infections due to *Mycobacterium avium* complex; clinical tuberculosis in the AIDS era and the role for preventive therapy; multiple drug resistance in tuberculosis; and intermittent and directly observed chemotherapy for tuberculosis.—Trop. Dis. Bull. **95** (1998) 726

Roche, P., Dockrell, H. and Brennan, P. Progress in research towards a world without leprosy. Report of a WHO meeting in Ethiopia, February 1998. Lepr. Rev. **69** (1998) 151–159.

A UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases meeting to discuss the future role of biomedical research in leprosy was held at the Armauer Hansen Research Institute in Addis Ababa, Ethiopia, on 27 and 28 February 1998. This was attended by more than 20 scientists from 10 countries, who met to discuss progress toward a world without leprosy.—Authors' Summary

Chemotherapy

Arunthathi, S. And Raju, S. Dapsone induced pulmonary eosinophilia without cutaneous allergic manifestations—an unusual encounter—a case report. Acta Leprol. **11** (1998) 3–5.

Dapsone is a drug of choice in the treatment of leprosy. In addition it is very useful in the treatment of many other dermatological conditions. The "dapsone-induced hypersensitivity" is not unknown. However, to the best of our knowledge, pulmonary eosinophilia induced by dapsone without any cutaneous allergic manifestation has not been reported in leprosy patients. Pulmonary eosinophilia (Loeffler's syndrome) induced by dapsone without cutaneous manifestation has been reported in a nonleprosy patient by Janier, *et al.* We report a case of pulmonary eosinophilia associated with dapsone therapy in a patient with lepromatous leprosy without allergic cutaneous manifestations and our experience in the management of this patient.—Authors' Abstract

Gadre, D. V., Talwar, V., Gupta, H. C. and Murthy, P. S. Effect of trifluoperazine, a potential drug for tuberculosis with psychotic disorders, on the growth of clinical isolates of drug resistant *Mycobacterium tuberculosis.* Int. Clin. Psychopharmacol. **13** (1998) 129–131.

The effect of the antipsychotic drug trifluoperazine (TFP) on the *in-vitro* growth of 50 clinical isolates of *Mycobacterium tuberculosis* was tested. Of these isolates, 29 were susceptible to all five of the antitubercular drugs (isoniazid, rifampin, streptomycin, ethambutol and pyrazinamide) and 21 were resistant to one or more of the five drugs. The minimum inhibitory concentration (MIC) of TFP was 4 μg/ml for 40% of both the susceptible (12/29) and resistant (8/21) isolates and 8 μg/ml for 55% (16/29) and 48% (10/21) of the susceptible and resistant isolates, respectively. Further analysis of the data for resistant isolates indicated that the MIC of TFP was 4 μg/ml and 16 μg/ml, respectively, for 50% (4/8) and 75% (6/8) of the isolates resistant to one drug only from isoniazid, streptomycin or pyrazinamide. Of the nine isolates resistant to two drugs, isoniazid and streptomycin, the MIC was 4 μg/ml for 33% (3/9) and 16 μg/ml for 80% (7/9). The MIC of TFP for two isolates resistant to the three drugs isoniazid, rifampin and streptomycin was 8 μg/ml for one and 32 μg/ml for the other. Of two isolates resistant to all five drugs, it is of interest to note that the MIC of TFP was only 4 μg/ml for one but 32 μg/ml for the other. Because the above MICs are for TFP as a single drug, it would be desirable to study the antitubercular activity of the serum of tuberculosis patients with psychotic problems receiving regular antitubercular therapy supplemented with TFP at its recommended and tolerated dose.—Authors' Abstract

Ishi, N., Sugita, Y., Sato, I. and Nakajima, H. Sparfloxacin in the treatment of leprosy patients. Int. J. Dermatol. **36** (1997) 619–621.

Seven men with leprosy visited the dermatology clinic at Yokohama City University Hospital, Japan, [date not given] and were entered into a trial of sparfloxacin

(SPFX), 100–200 mg daily for up to 1 year. Five patients were Japanese Brazilians or Paraguayan from South America, and 2 patients were Filipinos. Examination procedures included a detailed medical history, pretreatment, clinical examinations and body charting of the characteristic skin lesions, areas of anesthesia, and enlarged peripheral nerves. There were no deformities observed in any patient. All patients had presented with eruptions and neurological problems. The diagnosis of *Mycobacterium leprae* infection and its type were determined by the above examination, skin smears, and histopathological study, as well as the Mitsuda reaction. Following initiation of SPFX, erythema and swelling in all patients disappeared in only a few weeks, and new skin lesions did not appear in any case. In one of the patients from The Philippines, who had first presented in 1993, and had lepromatous leprosy, a bacterial index and a morphological index were determined. Bacilli in skin smears disappeared quickly and there was a dramatic morphological change as well as clinical improvement. One patient complained of neuralgia just after SPFX treatment, and this was clinically presumed to be type I reversal lepra reaction; this was the only adverse effect of the study.—Trop. Dis. Bull. **95** (1998) 731–732

Ladhani, S. Leprosy disabilities: the impact of multidrug therapy (MDT). Int. J. Dermatol. **36** (1997) 561–572.

The impact of MDT on leprosy disabilities is reviewed. Disabilities following infection, including prevalence and incidence; compliance, early diagnosis and rehabilitation; and disabilities following reactions and relapse, and psychosocial disabilities are discussed. It is concluded that until a suitable vaccine is available to prevent leprosy, MDT remains the best chance of controlling leprosy and the disabilities seen with the disease.—Trop. Dis. Bull. **95** (1998) 731

Li, B., *et al.* [Relapse of 46 cases of leprosy in Wuxi, Jiangsu.] China Lepr. J. **14** (1998) 87–88. (in Chinese)

In Wuxi City, Jiangsu, China, up to the year 1996 there have accumulatively been 1791 registered cases of leprosy, of which 1474 have been cured. During the period 1970 to 1996 relapse was detected in 46 cases (3.12%), i.e., 0.3%/year on average. The duration from cure to relapse was 5 to 231 months, averaging 5.57 years. Nine out of the relapsed cases have degraded from the TT pole to the LL pole, but five upgraded; 95.7% of the relapses were those who had taken dapsone monotherapy. The relapse rate was 6% in multibacillary and 2.3% in paucibacillary cases.—Authors' English Abstract

Li, H., *et al.* [Risk of relapse following FD MDT in leprosy.] China Lepr. J. **14** (1998) 69–74. (in Chinese)

During 1986 to 1995, 8307 leprosy patients [multibacillary (MB) 598 and paucibacillary (PB) 2326] had completed their fixed duration (FD) MDT and then follow up was done annually in 5 successive years in 59 counties of Yunnan, Guizhou and Sichuan. Among them, the mean relapse rate was 0.15 per 1000 person-years in MB and 0.55 per 1000 person-years in PB. There was no difference between the relapse rates of those who received dapsone (DDS) or DDS plus rifampin (RMP) (5111) and not (3196) before FD MDT. Five relapsed MB cases occurred 4 to 7 years after stopping treatment and five PB cases relapsed 4 to 5 years after stopping the drugs. Another six relapsed PB cases occurred after five-year follow up duration and were not calculated in the relapse rate. The majority of relapsed PB cases (6/11) should originally have been given the MB regimen, but used the PB one, and so the treatment was not enough for them. There were eight PB cases that became MB after relapse. Among 62 MB cases with irregular treatment four cases relapsed, being 6.5%. —Authors' English Abstract

Mao, Q., *et al.* [Effects of retreatment with MDT in persons cured of leprosy by using DDS monotherapy.] China Lepr. J. **14** (1998) 86–87. (in Chinese)

In Jiangyan City, Jiangsu, China, 1329 persons, who have been cured of leprosy with dapsone (DDS) monotherapy after a mean of 7.8 years and were paucibacillary (PB) in 1158 and multibacillary (MB) in 171, have received multidrug therapy (MDT) according to the PB regimen for 6 months for preventing relapse. And afterward during follow up of 1.5 to 12 years, 12 relapsed cases (0.9%) [PB 8 (0.69%) and MB 4 (2.34%)] were found, but among 1632 who did not receive MDT retreatment the relapse rate was as high as 2.75%, being 2.47% in 1457 PB cases and 2.34% in 175 MB ones. The authors thought that a 6-month retreatment with the PB regimen of MDT might reduce the relapse in people cured of leprosy with DDS monotherapy.—Authors' English Abstract

Shen, J., *et al.* [Effect of MINO on vitality of *M. leprae* in the body of MB leprosy patients.] China Lepr. J. **14** (1998) 74–75. (in Chinese)

Fourteen cases of multibacillary (MB) leprosy have taken minocycline 100 mg a day in 6 days of a week for 3 months, of which eight cases had skin biopsies taken before and in the months 1, 2 and 3 of treatment and inoculated into mouse foot pads for measurement of the drug's activity against *M. leprae.* The result showed that in the first month of treatment the inoculations of four cases had no growth and after month 2 all had no growth; suggesting very high antibacterial potency. The authors think that the drug can be routinely used as an alternative antileprosy drug.—Authors' English Abstract

Terencio de las Aguas, J. [Relapses in leprosy; personal experience.] Hansenol. Int. **22** (1997) 5–9. (in Spanish)

Thirty-one relapses were observed among 451 patients treated with sulfones and 15 of them were of the dimorphous form. The time elapsed between regression of lesions and the relapses range from 6 to 39 years. Only one relapsed case was observed among the patients treated with MDT/WHO regimens.—Author's English Summary

World Health Organization. Leprosy elimination campaigns—reaching every patient in every village. Wkly. Epidemiol. Rec. **72** (1997) 205–210.

This paper discusses the Leprosy Elimination Campaign (LEC), an initiative which aims to provide national leprosy elimination programs with additional external input to intensify elimination activities, with technical cooperation from WHO and other agencies. Its main objective is to detect leprosy cases that have remained undetected in the community, and to promptly put them onto multidrug therapy (MDT). Of an estimated 800,000 backlog cases, 650,000 are expected to be diagnosed and treated through LECs during 1996–2000, with elimination activities particularly intensified during the top 16 leprosy-endemic countries (accounting for 95% of those affected). Results are presented of LECs carried out during 1996 in Cambodia (2), Indonesia, Nepal, Nigeria, and The Philippines (2), the duration of each campaign being 3–4 months. A total of 2188 cases were detected by the seven campaigns, more than double the number of cases detected in these areas during 1995. The multibacillary (MB) proportion among the new cases detected by the campaigns ranged between 47% and 65% and the proportion of grade 2 disabilities ranged between 12% and 33%, showing that the campaigns were able to detect hidden leprosy cases of consequence. All cases detected during the campaigns were treated with WHO/MDT. Additionally, reorientation training was provided to around 1000 local health workers to improve MDT services in their respective areas, and simple task-oriented training was given to >9000 volunteers from the communities where LECs were carried out to increase community awareness and to help patients to reach the nearest health center for regular treatment.—Trop. Dis. Bull. **95** (1998) 731

World Health Organization. Progress towards leprosy elimination. Wkly. Epidemiol. Rec. **72** (1997) 165–171.

This report records the progress that has been made toward the goal of worldwide elimination of leprosy as a public health

problem, updating figures published in June 1996. Data is presented for each WHO region showing numbers of estimated and registered leprosy cases in 1996–1997, detection rates, and registered cases of leprosy and coverage with multidrug therapy (MDT). Detailed information is presented for the top 16 countries endemic for leprosy and for countries with >100 registered cases. The number of countries showing prevalence rates for leprosy above 1 per 10,000 population decreased from 122 in 1995 to 55 at the beginning of 1997. A total of 67 countries have reduced the leprosy prevalence to below 1 per 10,000 population. At the beginning of 1997 it was estimated that there were around 1,150,000 leprosy cases worldwide, and of these, 888,340 were registered for treatment. Only a small reduction in the number of registered cases (4%) was noted between 1996 and 1997; the global prevalence of registered cases, which was constantly decreasing over the previous 10 years, was still around 1.6 per 10,000 population. In the 16 major endemic countries, which represent 91% of the global leprosy problem, the prevalence rate was still 4.3 per 10,000, indicating that to eliminate leprosy as a public health problem, additional efforts will be needed. Regarding detection of leprosy cases, around 555,000 cases were detected during 1996 as notified by 79 countries. Allowing for some endemic countries which had not sent in information at time of reporting, the global detection was therefore estimated at 566,000 (9.8 per 100,000 population). Around 535,000 cases (95%) were detected in the 16 major endemic countries, and 73% of the newly detected cases were living in India alone. Among the newly detected cases, >85,000 (16%) were children, around 17,000 (31%) were multibacillary (MB) cases and around 30,000 (5.5%) were showing severe disabilities at time of diagnosis. Concerning progress with MDT, during 1996 around 1.4 million patients were treated with MDT and >555,000 were cured.—Trop. Dis. Bull. **95** (1998) 732

World Health Organization. Shortening duration of treatment of multibacillary leprosy. Wkly. Epidemiol. Rec. **72** (1997) 125–128.

Information on cohorts of multibacillary (MB) leprosy patients, including MB skin-smear positive patients, who started multiple drug therapy (MDT) between 1986 and 1990 was collected by WHO in collaboration with national leprosy programs (including national leprosy institutions) in order to determine the outcome of patients who had been unable to complete the full course of treatment of 2 years. Any patient who was not able to complete 24 doses of MDT was classified as a defaulter and was actively traced, their clinical and bacteriological status being assessed and compared with their status recorded at time of starting MDT. This analysis indicated that, in terms of treatment failure or relapse, the fate of defaulters taking less than 12 months' treatment is quite favorable, reflected by observation of continued clinical and bacteriological improvement at time of retrieval. It is suggested that even a limited number of doses of MDT are beneficial to the individual patient in eliminating nearly all the viable *Mycobacterium leprae,* in curing the disease and in preventing disease transmission, and that a shorter WHO/MB MDT regimen of 12 months could be sufficient for practically all MB patients.—Trop. Dis. Bull. **95** (1998) 732

Clinical Sciences

Arunthathi, S., Ebenezer, L. and Kumuda, C. Reversal reaction, nerve damage and steroid therapy in three multibacillary HIV positive patients. Lepr. Rev. **69** (1998) 173–177.

The progress of three Indian HIV-positive patients with multibacillary leprosy has been recorded.—Authors' Abstract

Burdick, A. E., Lehrer, K. A. and Barquin, L. Use of eutectic mixture of local anesthetics: an effective topical anesthetic for slit-smear testing of patients with Hansen's disease. J. Am. Acad. Dermatol. **37** (1997) 800–802.

This double-blind, placebo-controlled, randomized trial in the state of Florida, U.S.A., was conducted [date not given] to determine the efficacy of a eutectic mixture of local anesthetics ([EMLA] lidocaine 2.5% and prilocaine 2.5%), applied under occlusion as cream, in providing dermal anesthesia for slit-smear testing in leprosy patients. Nineteen adult leprosy patients, 3 with newly diagnosed disease and 16 with established multibacillary (borderline lepromatous and lepromatous) disease (11 male; 4 black and 15 white or Hispanic) were studied, with each participant acting as their own control. Slit-smear testing was performed on the right and left earlobes, elbows and knees. One hr before the procedure either EMLA or placebo cream was applied to the patient's right earlobe, elbow and knee, and the other cream was applied to the left earlobe, elbow and knee. Patients completed 6 visual analog scales (ranging from 0 mm, indicating no pain, to 50 mm, indicating severe pain) to quantify their pain for each of the 6 slit-smear testing procedures. Patients reported that slit-smear tests performed on elbows and knees treated with the EMLA cream were less painful than those on corresponding sites treated with placebo (mean visual analog scores were 9.32 mm with EMLA treatment and 16.95 mm with placebo for elbows ($p < 0.01$) and 10.1 mm for EMLA treatment and 23.3 mm with placebo for knees ($p < 0.05$)). On the earlobes, the difference between the means of pain scores for the placebo and the EMLA cream was not significant. There was no significant difference by paired *t* test between any of the bacillary or morphological index means from any corresponding EMLA and placebo sites.—Trop. Dis. Bull. **95** (1998) 607–608

Conejo-Mir, J. S., Artola-Igarza, J. L., Garciandia, C., Linares-Barrios, M. and Navarrete, M. Hemicorporal distribution of lepromatous leprosy in a patient with hemiplegia. Clin. Infect. Dis. **27** (1998) 212–213.

This is a brief report of a 75-year-old male who presented with multiple papular and nodular lesions confined to the left side of his body. The patient gave a history of a stroke 26 years previously with a residual left hemiplegia. He had a history of treatment for diabetes mellitus for the last 35 years. Biopsies of the lesions showed lepromatous leprosy. Slit-skin smears were positive for acid-fast bacilli from the left arm but negative from the right arm. The authors point out that the limbs of patients with paresis are 1°C to 5°C cooler than the limbs of healthy individuals. This case seems to be a clinical example of the affinity of *Mycobacterium leprae* for cooler parts of the body.—RCH

dePaula, M., Saiz, L. C., Gonzalez Revalderia, J., Pascual, T., Alberola, C. and Miravalles, E. Rifampicin causes false-positive immunoassay results for urine opiates. Clin. Chem. Lab. Med. **36** (1998) 241–243.

The treatment of tuberculosis usually includes the antibiotic rifampin, especially in patients with concomitant human immunodeficiency virus infection. Some of these patients are in withdrawal therapy for drug abuse. When opiate screening is carried out in patients receiving rifampin, false-positive results are detected with the kinetic interaction of microparticles in solution method. We evaluated this interference in a Cobas-Integra analyzer and found a 12% crossreactivity of rifampin for antibiotic concentrations ranging from 0.19 to 6.08 µmol/l (156 to 5000 µg/l). This effect is not explained by the color of the rifampin solutions. Calculations assuming first order kinetics of elimination show that more than 18 hr after a single oral dose of 600 mg of rifampin, a false-positive result for opiates could be obtained. This indicates that the risk of a false-positive result must always be considered when urine samples from these patients are analyzed.—Authors' Abstract

Ebenso, B. E. Seizures following chloroquine treatment of type II lepra reaction: a case report. Lepr. Rev. **69** (1998) 178–181.

A case of tonic-clonic seizures following chloroquine treatment for leprosy reactions in a Nigerian male is reported. Seizures were controlled with phenytoin sodium capsules. A causal relationship between the seizures and chloroquine is suggested. There have been no previous reports of this adverse reaction in leprosy patients receiving chloroquine for treatment of reactions. The author recommends that chloroquine be used with caution especially in patients with seizures.—Author's Summary

Feng, Z. [Leprosy control in Chencen town of Shunde County, Guangdong.] China Lepr. J. **14** (1998) 85–86. (in Chinese)

In Chencen of Shunde County located in the Zhujiang Delta, 18 km away from Guangzhou, with a population of seventy-odd thousand, 124 leprosy patients were accumulatively found by the end of 1996, of whom 106 have been cured, 16 died, and 2 remain under MDT. The prevalence decreased from 1.5‰ to 0.02‰. Since 1958 one doctor has been appointed to the leprosy control post in the town hospital, who is under the leadership of the head of the hospital and guidance of the County's leprosy control station, being responsible for leading village health workers in controlling leprosy, and so an effective network for leprosy has been made up in the whole country.—Author's English Abstract

Huang, S. [Knowledge and behavior on health care among leprosy patients.] China Lepr. J. **14** (1998) 82–83. (in Chinese)

Analysis of the knowledge and behavior on health care in leprosy patients showed that among them 42.79% had no correct recognition of the infectivity of leprosy, 32.83% did not know how to prevent disability, and 39.86% of those with disability did not know how to rehabilitate, and among those who knew it only 25.58% were practicing it. There were 0.07% of the patients who have been increasing nutrient for their disease. The perceptivity was associated with cultural level. The author pointed out that leprosy patients acquire the knowledge of the disease only through the doctor's explanation and so variegated health education should be launched, especially through village health workers.—Author's English Abstract

Monteiro, L. G., Campos, W. R., Orefice, F. and Grossi, M. A. F. Study of ocular changes in leprosy patients. Indian J. Lepr. **70** (1998) 197–202.

In this study, 997 leprosy patients were examined, 528 of them with lepromatous leprosy (53%), 199 with borderline leprosy (20%), 167 with tuberculoid leprosy (16%) and 103 (10.3%) with indeterminate leprosy. Changes in the ocular bulb were noted in 314 patients (31.5%) especially in those with lepromatous leprosy. These alterations were greater with increasing age of the patient and length of disease. Severe ocular lesions were rare, probably due to previous systemic treatment. The "pearls" in the fundus of the eye resulting from leprosy were also studied.—Authors' Abstract

Silva, E. A., Rubio, E. M. and Ura, S. [An investigation of ABH antigens in erythrocytes and saliva of leprosy patients.] Hansenol. Int. **22** (1997) 44–49. (in Portuguese)

The few studies that have already been published about the determination of secretor phenotypes of the salivary ABH antigens of leprosy patients have shown there is not a significant correlation between these substances and susceptibility to the disease. In the present study 74 patients were evaluated (27 lepromatous, 23 tuberculoid and 24 borderline) about the presence of ABH antigens on the erythrocytes and in the saliva by techniques of agglutination reaction in tube and inhibition of agglutination. The frequency of ABO blood groups and secretor/nonsecretor phenotype in these patients and in the control group were: O = 40.5% (49%); A = 41.9% (36.5%); B = 10.8% (10.9%); AB = 6.8% (3.6%); secre-

tor = 68.9% (82.5%) and nonsecretor = 31.1% (17.5%).

By the analysis of the results in leprosy patients, significant differences were not observed when comparing the distribution of ABH antigens, on the erythrocytes and in the saliva, with the control group.—Authors' English Summary

Tan, X. [Leprosy among children 14 years old in Guangzhou.] China Lepr. J. **14** (1998) 84–85. (in Chinese)

In Guangzhou, China, there were a total of 7415 registered cases of leprosy by the end of 1995, of whom 358 were children with ages less than 14 years, including 213 boys and 145 girls, and MB 108 (28.2%) and PB 257 (71.8%). Among them those infected in their families were 99 cases (27.7%). On diagnosis, the disease durations were 1 to 108 months, 79.6% of their skin lesions were in exposed sites and 14 cases (11.5%) had visible disabilities. Most of the children (80.8%) were detected passively; 326 cases have been cured, for a mean treatment duration of 6.6 years, of whom 27 relapsed (8.3%). Since 1990 no child suffering from leprosy was seen, which, the author thinks, may be because of the wide use of BCG for prevention of TB since the 1960s.—Author's English Abstract

Tang, X. [Changes in the cornea and of vision in leprosy patients.] China Lepr. J. **14** (1998) 89–90. (in Chinese)

Since 1994 to 1996, 824 persons who have or who had had leprosy were examined for oculopathy. The results showed that 507 eyes had lagophthalmos, ectropion, corneal hypesthesia or coexistence of some of them, and in them 120 eyes had keratopathy (24%), with hypopsia of 0.2 to 0.7. And in 1141 eyes without the above oculopathy 34 had keratopathy (2.9%) with the vision of 0.82 on an average. The author pointed out that the ectropion showed greater menace to the vision than did lagophthalmos.—Author's English Abstract

Trifilo, M. O., Belone, A. F. F. and Fleury, R. N. [Bacilloscopic evaluation in Virchowian hanseniasis (study of 60 necropsies).] Hansenol. Int. **22** (1997) 10–19. (in Portuguese)

In the literature there are reports that the proliferation of *Mycobacterium leprae* in viscera could occur even in the absence of proliferation at neuro-cutaneous level, and this fact could allow reactivation of leprosy. We analyzed 60 necropsies of lepromatous leprosy patients to compare and contrast from several visceral sites with those from skin and peripheral nerves. The necropsies were divided in groups according to results of the last bacilloscopy done while the patients were alive: active lepromatous and in progression–15 necropsies; active lepromatous and in regression–17 necropsies; inactive lepromatous–28 necropsies. In some necropsies, the visceral bacterial index (BI) overpassed the same index at the neuro-cutaneous level, and in 3 could not be appraised because technical and legal limitation prevented us from collecting skin and peripheral nerve fragments from various sites. Nevertheless, some reports suggest that the bacilloscopy of skin drainage lymph nodes can be an estimate of cutaneous bacilloscopy. In some of the visceral necropsies examined the BI surpassed the same index in axillary lymph nodes. Except for the axillary lymph nodes, the highest visceral BIs were found in the larynx, testicle and pharynx, with predominance of the first site. This may reinforce the relationship between the tissue temperature and bacillary proliferation ability, suggesting that the larynx presents conditions of adaptation and proliferation of *M. leprae* similar to the nasal mucous, which is the main way of bacillary shedding.—Authors' English Summary

Wang, B., *et al.* [A survey of the oculopathy in 1897 cases of leprosy.] China Lepr. J. **14** (1998) 92–94. (in Chinese)

Among 1897 persons with ages of 38 to 71 years, who have and had leprosy in 96 and 1801, respectively, and were treated with DDS monotherapy in 1287 and with MDT in 610, including 1486 male and 441

female, a survey of oculopathy showed that there were madarosis in 1029 (73.3%), trichiasis in 689 (49.1%), ectropion in 575 (40.9%), lagophthalmos in 340 (24.2%), blocking of lacrimal duct in 258 (18.3%), conjunctivitis in 502 (35.7%), corneal hypoesthesia in 432 (30.7%), corneal ulcer in 277 (19.7%), keratitis in 281 (20%), leucoma in 41 (2.9%), scleritis in 116 (8.2%), iridocyclitis in 483 (34.4%), cataracts in 48 (3.4%), and low vision in 430 eyes of 379 persons (20%) and blindness in 180 persons (9.4%) with unilateral side in 93 and bilateral in 87. The oculopathy mostly occurred (63.2%) 5 to 15 years after the onset of leprosy. The oculopathy was 5.2% in 117 persons who can do self-care and 35.8% in 446 persons who cannot do so. The causes of blindness mainly were iridocyclitis and keratopathy.—Authors' English Abstract

Immuno-Pathology

Baldwin, S. L., D'Souza, C., Roberts, A. D., Kelly, B. P., Frank, A. A., Lui, M. A., Ulmer, J. B., Huygen, K., McMurray, D. M. and Orme, I. M. Evaluation of new vaccines in the mouse and guinea pig model of tuberculosis. Infect. Immun. **66** (1998) 2951–2959.

The results of this study provide the first evidence that two completely separate vaccine approaches, one based on a subunit vaccine consisting of a mild adjuvant admixed with purified culture filtrate proteins and enhanced by the cytokine interleukin-2 and the second based on immunization with DNA encoding the Ag85A protein secreted by *Mycobacterium tuberculosis,* could both prevent the onset of caseating disease, which is the hallmark of the guinea pig aerogenic infection model. In both cases, however, the survival of vaccinated guinea pigs was shorter than that conferred by *M. bovis* BCG, with observed mortality of these animals probably due to consolidation of lung tissues by lymphocytic granulomas. An additional characteristic of these approaches was that neither induced skin-test reactivity to commercial tuberculin. These data thus provide optimism that development of nonliving vaccines which can generate long-lived immunity approaching that conferred by the BCG vaccine is a feasible goal.—Authors' Abstract

Bottasso, O., Merlin, V., Cannon, L., Cannon, H., Ingledew, N., Keni, M., Hartopp, R., Stanford, C. and Stanford, J. Studies of vaccination of persons in close contact with leprosy patients in Argentina. Vaccine **16** (1998) 1166–1171.

A total of 670 adults living or working with leprosy patients were examined for a BCG vaccination scar and skin-tested with four new tuberculins. Based on the results 513 were vaccinated; 65 with bacille Calmette Guerin (BCG) alone, 66 with BCG plus killed *Mycobacterium vaccae* and 382 with killed *M. vaccae* alone. Skin-testing was repeated 2–3 years later on 344 subjects, when all three vaccines were found to have been highly successful in increasing responses to tuberculin and leprosin A ($p < 0.0005$) with increased immune recognition of common and species-specific antigens. Mean diameters of induration to each skin test were greatest in recipients of BCG alone ($p < 0.05$), which suggests that better immuno-regulation occurs after receiving vaccines that incorporate *M. vaccae.* The results suggest 10^8 *M. vaccae* alone might prove a valuable future vaccine, which would not require selective pre-vaccination procedures.—Authors' Abstract

Brasil, M. T. L. R. F., de Oliveira, L. R., de Mello, C. S., Nakamura, P. M., Manini, M. P., Steiner, D. and Rotta, O. [A study of the sensitivity and specificity of the anti-PGL-I ELISA.] Hansenol. Int. **22** (1997) 35–43. (in Portuguese)

Serum tests for leprosy diagnosis, using phenolic glycolipid-I, considered to be a specific antigen of *M. leprae,* has brought

about some possibilities to the study of the epidemiological behavior of the disease. A study was performed to evaluate the sensibility and specificity of an ELISA anti-PGL-I test using Cuban material and techniques (UMELISA). Eighty-four patients and a control group with 112 persons were tested.

The sensibility of the test was larger for the multibacillary patients with higher levels for the case classified as lepromatous (L) followed by the borderline (B) ones. In the multibacillary group, considering a 0.200 cut-off level, it was observed that only 75.0% of the lepromatous patients and 50.0% of the borderline patients tested positive. For a 0.300 cut-off level, only 64.3% of the L patients and 40.0% of the B patients still tested positive. The undetermined form patients presented a larger rate of positive results than the tuberculoid form, and that may be a hint of a polarization of some cases of the multibacillary forms. The specificity of the test was 87.5% at the 0.200 cut-off level and 99.1% at 0.300. This test, as some others, presented high specificity and low sensibility, resulting in a large percentage of false-negative tests.

Since leprosy is a disease with few serum tests, it is found that further technological development of this test should be stimulated, although its indiscriminate use for large-scale screening of the general population is not recommended. This way, advances in the research of more sensitive and specific tools for early diagnosis could be reached allowing for efficient intervention on the transmission chain of the disease.—Authors' English Summary

Gupta, P. N. and Pal, N. K. Is PGL-I also present in *Leishmania donovani* promastigotes? Indian J. Lepr. **70** (1998) 161–164.

A soluble antigen complex (SAC) derived from the ruptured promastigotes of *Leishmania donovani* parasites (LD-SAC) was used for complement fixation test (CFT) in leprosy. Cases of tuberculoid and borderline tuberculoid leprosy, post-kala-azar dermal leishmaniasis (TT, BT, PKDL) and control sera gave negative CFT. Smear-positive cases of borderline (BB, BL) and lepromatous (LL) leprosy and drug-resisting cases of pulmonary tuberculosis gave positive CFT; smear-negative cases of LL leprosy sera also gave positive CFT. Sera of smear-negative inactive LL patients contained only PGL-I and PDIM antigens for a long time after they become inactive. Therefore, the positive CFT in inactive LL makes us suspect whether PGL-I is present in LD promastigotes.—Authors' Abstract

Haslett, P. A. J., Corral, L. G., Albert, M. and Kaplan, G. Thalidomide costimulates primary human T lymphocytes, preferentially inducing proliferation, cytokine production, and cytotoxic responses in the CD8+ subset. J. Exp. Med. **187** (1998) 1885–1892.

The efficacy of thalidomide (alpha-phthalimido-glutarimide) therapy in leprosy patients with erythema nodosum leprosum is thought to be due to inhibition of tumor necrosis factor alpha. In other diseases reported to respond to thalidomide, the mechanism of action of the drug is unclear. We show that thalidomide is a potent costimulator of primary human T cells *in vitro*, synergizing with stimulation via the T-cell receptor complex to increase interleukin 2-mediated T-cell proliferation and interferon gamma production. The costimulatory effect is greater on the CD8+ than the CD4+ T-cell subset. The drug also increases the primary CD8+ cytotoxic T-cell response induced by allogeneic dendritic cells in the absence of CD4+ T cells. Therefore, human T-cell costimulation can be achieved pharmacologically with thalidomide, and preferentially in the CD8+ T-cell subset.—Authors' Abstract

Kang, B. K. and Schlesinger, L. S. Characterization of mannose receptor-dependent phagocytosis mediated by *Mycobacterium tuberculosis* lipoarabinomannan. Infect. Immun. **66** (1998) 2769–2777.

The macrophage mannose receptor (MR) along with complement receptors mediates phagocytosis of the *Mycobacterium tuber-*

culosis virulent strains Erdman and H37Rv. We have determined that the terminal mannosyl units of the *M. tuberculosis* surface lipoglycan, lipoarabinomannan (LAM), from the Erdman strain serve as ligands for the MR. The biology of the MR (receptor binding and trafficking) in response to phagocytic stimuli is not well characterized.

This study analyzes the MR-dependent phagocytosis mediated by Erdman LAM presented on a 1-μm-diameter phagocytic particle. Erdman LAM microspheres exhibited a time- and dose-dependent rapid increase in attachment and internalization by human monocyte-derived macrophages (MDMs). In contrast, internalization of LAM microspheres by monocytes was minimal. Microsphere internalization by MDMs was visualized and quantitated by immunofluorescence and confocal and electron microscopy and resembled conventional phagocytosis. Phagocytosis of LAM microspheres by MDMs was energy, cytoskeleton, and calcium dependent and was mannan inhibitable. Trypsin treatment of MDMs at 37°C, which depleted surface and recycling intracellular pools of the MR, reduced the subsequent attachment of LAR I microspheres. Trypsin treatment at 4°C allowed for subsequent recovery of LAM microsphere phagocytosis at 37°C by recycled MRs. Pretreatment of MDMs with cycloheximide influenced LAM microsphere phagocytosis to only a small extent, indicating that MR-dependent phagocytosis of the microspheres was occurring primarily by preformed recycled receptors. This study characterizes the requirements for macrophage phagocytosis of a LAM-coated particle mediated by the MR. This model will be useful in further characterization of the intracellular pathway taken by phagocytic particles coated with different LAM types in macrophages following ingestion.—Authors' Abstract

Lewisohn, D. M., Alderson, M. R., Briden, A. L., Riddell, S. R., Reed, S. G. and Grabstein, K. H. Characterization of human CD8+ T cells reactive with *Mycobacterium tuberculosis*-infected antigen-presenting cells. J. Exp. Med. **187** (1998) 1633–1640.

Previous studies in murine models, including those using the beta 2 microglobulin knockout mouse, have suggested an important role for CD8+ T cells in host defense to *Mycobacterium tuberculosis* (Mtb). At present, little is understood about these cells in the human immune response to tuberculosis. This report demonstrates the existence of human Mtb-reactive CD8+ T cells. These cells are present preferentially in persons infected with Mtb and produce interferon gamma in response to stimulation with Mtb-infected target cells. Recognition of Mtb-infected cells by these CD8+ T cells is restricted neither by the major histocompatibility complex (MHC) class I A, B, or C alleles nor by CD1, although it is inhibited by anti-MHC class I antibody. The Mtb-specific CD8+ T cells recognize an antigen which is generated in the proteasome, but which does not require transport through the golgi-ER. The data suggest the possible use of nonpolymorphic MHC class Ib antigen presenting structures other than CD1.—Authors' Abstract

Mukhopadhyay, A., Panda, A. K. and Pandey, A. K. Leprosy vaccine: influence of dissolved oxygen levels on growth of a candidate strain (*Mycobacterium w*), and storage stability of the vaccine. Vaccine **16** (1998) 1344–1348.

The growth of *Mycobacterium w*, a candidate strain for leprosy vaccine in submerged culture, was inhibited by the presence of over 40% oxygen saturation in the medium.

Intracellular levels of superoxide dismutase and catalase were very low in the beginning. However, under controlled oxygenation, these levels increased with time.

The augmentations of these antioxidant enzymes were associated with the elevated oxygen consumption by the culture. By maintaining the oxygen level below 20% during a 6-day culture, it was possible to grow *Mycobacterium w* in five production batches up to a cell density of $3.7 \pm 0.70 \times 10^9$ bacilli ml^{-1}. The shelf-life of the vaccine produced in different batches was more than 2 years, both at 4°C and at 26°C. This provides a cost effective, unit culture tech-

nology for the production of this candidate leprosy vaccine from a nonpathogenic organism, which will facilitate widespread use of the vaccine.—Authors' Abstract

Oddo, M., Renno, T., Attinger, A., Bakker, T., MacDonald, H. R. and Meylan, P. R. A. Fas ligand-induced apoptosis of infected human macrophages reduced the viability of intracellular *Mycobacterium tuberculosis.* J. Immunol. **160** (1998) 5448–5454.

Mycobacterium tuberculosis-specific cytolytic activity is mediated mostly by CD4+ CTL in humans. CD4+ CTL kill infected target cells by inducing Fas (APO-1/CD95)-mediated apoptosis. We have examined the effect of Fas ligand (Fas-L)-induced apoptosis of human macrophages infected *in vitro* with *M. tuberculosis* on the viability of the intracellular bacilli. Human macrophages expressed Fas and underwent apoptosis after incubation with soluble recombinant Fas. In macrophages infected either with an attenuated (H37Ra) or with a virulent (H37Rv) strain of *M. tuberculosis,* the apoptotic death of macrophages was associated with a substantial reduction in bacillary viability. TNF-induced apoptosis of infected macrophages was coupled with a similar reduction in mycobacterial viability, while the induction of nonapoptotic complement-induced cell death had no effect on bacterial viable counts. Infected macrophages also showed a reduced susceptibility to Fas-induced apoptosis correlating with a reduced level of Fas expression. These data suggest that apoptosis of infected macrophages induced through receptors of the TNF family could be an immune effector mechanism not only depriving mycobacteria from their growth environment but also reducing viable bacterial counts by an unknown mechanism. On the other hand, interference by *M. tuberculosis* with the Fas system might represent an escape mechanism of the bacteria attempting to evade the effect of apoptosis.—Authors' Abstract

Partida Sanchez, S., Favila Castillo, L., Pedraza Sanchez, S., Gomez Melgar, M., Saul, A., Estrada Parra, S. and Estrada Garcia, I. IgG antibody subclasses, tumor necrosis factor and IFN-gamma levels in patients with type II lepra reaction on thalidomide treatment. Int. Arch. Allergy Immunol. **116** (1998) 60–66.

A group of 9 Mexican lepromatous leprosy patients was studied at the beginning of a type 2 reaction (erythema nodosum leprosum, ENL) and after 1 or 2 months of thalidomide treatment. ENL patients at the onset of the reaction had slightly higher amounts of anti-*Mycobacterium leprae* IgG1 and IgG2 antibodies compared to similar lepromatous patients that did not develop ENL.

Neither these antibody levels nor IgM and the other IgG subclasses were importantly modified after thalidomide treatment. Serum TNF was significantly higher in the patients that developed ENL compared to those that did not develop the reaction. TNF levels were slightly decreased after 1 month of thalidomide treatment and significantly decreased after 2 months of treatment. Serum IFN-gamma was significantly lower in patients at the onset of ENL and was increased after 1 and 2 months of thalidomide treatment.—Authors' Abstract

Ramanathan, V. D., Thyagi, P., Ramanathan, U., Katoch, K. and Ramu, G. A sequential study of circulating immune complexes, complement and immunoglobulins in borderline tuberculoid leprosy patients with and without reactions. Indian J. Lepr. **70** (1998) 153–160.

Sequential estimates of the levels of circulating immune complexes (CIC), complement catabolic fragment C3d, complement-mediated immune complex solubilization (CMS) and immunoglobulins were made in 24 newly diagnosed patients with borderline tuberculoid leprosy over a 20-month period after initiation of chemotherapy. Fourteen of these patients had not suffered from reversal reactions either at the time of presentation or during the follow-up period. The levels of CIC were elevated in them from the third to the eleventh month after starting chemotherapy and immuno-

globulin G (IgG) levels were elevated up to 8 months. the concentrations of C3d and immunoglobulins A (IgA) and M (IgM) were normal in these patients. The other 10 patients had reversal reactions at the time of diagnosis which subsided by the third month after starting treatment. They did not have reversal reactions later. The levels of CIC and IgG were elevated and those of CMS were depressed throughout the study period. Serum C3d level was initially elevated but came down to normal by the third month while IgA and IgM levels were within normal limits. The relevance of these findings to the genesis of reversal reaction is discussed in this communication. —Authors' Abstract

Singh, N., Bhatia, A., Arora, V. K. and Bhattacharya, S. N. Fine-needle aspiration cytology of lepromatous leprosy. Lepr. Rev. **69** (1998) 145–150.

A prospective study correlating cytopathology with clinical morphology and histopathology in 22 patients with lepromatous leprosy was performed. Aspirates were taken from skin lesions in all patients. Lymph node aspirates were also performed in four patients with lymphadenopathy. Fine-needle aspirates yielded sufficient cellular material with excellent preservation of morphological detail. Diagnosis and correlation with bacterial index, clinical and histopathological findings was possible in all patients. In addition, the two patterns, partial and diffuse, of lymph node involvement could be recognized. Fine-needle aspiration cytology is a simple method for the laboratory assessment of leprosy.—Authors' Summary

Tantawichien, T. Cytokines and mycobacterial infections. J. Infect. Dis. Antimicrob. Agents **14** (1997) 41–46.

In this overview, the role of cytokines in protective immunity to mycobacterial infections is examined using evidence of the interaction between mycobacteria and macrophages, and the role of lymphocytes and natural killer cells. The administration of tumor necrosis factor and granulocyte macrophage colony-stimulating factor in experimental models and the use of interleukin-2 and interferon gamma in multidrug treatment for leprosy are discussed. It is concluded that new knowledge of cytokine networks may lead to the development of novel therapeutic approaches using recombinant cytokines in mycobacterial infections and in those with other intracellular pathogens.—Trop. Dis. Bull. **95** (1998) 603

Trao, V. T., Huong, P. L. T., Thuan, A. T., Anh, D. D., Trach, D. D., Rook, G. A. W. and Wright, E. P. Changes in cellular response to mycobacterial antigens and cytokine production patterns in leprosy patients during multiple drug therapy. Immunology **94** (1998) 197–206.

Changes in *Mycobacterium leprae*-induced lymphoproliferative responses and mediator release by leprosy patients' lymphocytes were followed during multiple drug therapy (MDT). At the time of diagnosis, multibacillary (MB) patients who did not develop reactions responded to both sonicated *M. leprae* and synthetic disaccharide coupled to bovine serum albumin (ND-BSA) antigens, but those who would later develop reactions did not respond, even in the presence of added cytokines. The paucibacillary (PB) group initially had high responses to sonicated *M. leprae* but no response to ND-BSA, even in the presence of added cytokines. In the first year of treatment, the supernatants of PB patients' cell cultures contained factors that enhanced the phytohemagglutinin (PHA) response of normal cells. In contrast, those MB patients who did not develop reactions at a later stage produced culture supernatants that were inhibitory. Interestingly, the MB patients who later developed reactions during treatment, and did not initially respond to *M. leprae,* produced supernatants containing enhancing factors, like those of the PB group.

Later on in the treatment, all patients had the same patterns: when response to *M. leprae* decreased from its highest level, inhibitory factors were produced. Further studies revealed that the supernatants which inhibited the PHA response of normal cells

contained the active form of transforming growth factor-beta[1] (TGF-beta[1]), whatever the disease type or treatment status of the donor. These TGF-beta[1] levels correlated directly with the degree of inhibition. Similarly, supernatants that neither inhibited nor enhanced PHA responses contained the highest levels of interleukin-10 (IL-10), while those from treated patients that enhanced contained the lowest levels of IL-4 and interferon-gamma. These cytokine correlations transcended the conventional disease classification, and imply that all patients pass through a sequence of patterns of immune response during treatment. These treatment-induced changes may explain occasional reports of response patterns at variance with the "immunological spectrum" of leprosy.—Authors' Abstract

Triccas, J. A., Roche, P. W. and Britton, W. J. Specific serological diagnosis of leprosy with a recombinant *Mycobacterium leprae* protein purified from a rapidly growing mycobacterial host. J. Clin. Microbiol. **36** (1998) 2363–2365.

In this report we demonstrate the utility of a monoclonal antibody inhibition enzyme-linked immunosorbent assay based on the *Mycobacterium leprae* 35-kDa protein, purified from the rapidly growing host *M. smegmatis*, for the serodiagnosis of multibacillary leprosy. The assay proved highly specific (97.5%) and sensitive (90%) and compared favorably with two other established methods routinely utilized for leprosy serodiagnosis.—Authors' Abstract

Villahermosa, I. G., Abalos, R. M., Walsh, D. S., Fajardo, T. T. and Walsh, G. P. Recombinant interleukin-2 in lepromatous leprosy lesions: immunological and microbiological consequences. Clin. Exp. Dermatol. **22** (1997) 134–140.

Seven patients with lepromatous leprosy (LL) were inoculated with recombinant interleukin-2 (rIL-2) at five lesional sites on the back, four sites receiving one dose of 10 μg and biopsy specimens being obtained on 4 consecutive days after the injection. At the 5th site, rIL-2 was instead administered over several days, three patients receiving a total dose of 40 μg and four patients 150 μg, while biopsy specimens from this site were obtained 7, 14 and 21 days after the first injection. Most injection sites developed features of a delayed-type hypersensitivity reaction, namely, erythema and induration at the injection site, infiltrates rich in T-helper cells, monocytes, and Langerhans' cells and, at sites receiving higher doses, multinucleated Langhans' giant cells and epithelioid granulomas. In some patients, there were favorable shifts in histological classification or small changes in bacterial load. Low doses of rIL-2 injected into LL lesions rapidly enhance cellular immunity and may alter the histological classification or bacterial load at the injection site.—Authors' Abstract

Microbiology

Attia, S., Sheperd, V. E., Rosenblatt, M. N., Davidson, M. K. and Hughes, J. A. Interaction of oligodeoxynucleotides with mycobacteria: implications for new therapeutic strategies. Antisense Nucl. Acid Drug Develop. **8** (1998) 207–214.

The use of synthetic oligonucleotides (ONs) to systematically address new pharmacologic targets in mycobacteria would enhance the introduction of new molecular targets for drug intervention. Oligonucleotides' mechanism of action allows researchers to pursue the importance of particular proteins without the requirement of having purified samples. For this approach to be effective, mycobacteria must be able to transport ONs to their cytoplasm, and if this is not the case, the agents must be otherwise delivered. In this report, we characterize the ability of phosphorothioate (PS) and phosphorodiester (PD) ONs to interact with both *Mycobacterium smegmatis* and *M. tuberculosis*. In addition, the use of de-

livery enhancer compounds, ethambutol and PAMAM dendrimer, was evaluated on the ON-mycobacteria interaction. ON interaction was demonstrated to be concentration-dependent, suggesting a possibly active component of the oligonucleotide and bacteria interaction. ON interaction could be increased by the coincubation of the bacteria with the delivery adjuvants. Treatment with ethambutol or dendrimers (fourth generation) was demonstrated to increase ON interaction with both species of mycobacteria although not to the same extent. The results of these preliminary experiments indicate that through use of the proper delivery adjuvant, ON interactions with mycobacteria can be increased. These findings may have implications for probing future antimycobacterial therapeutic targets.—Authors' Abstract

Burman, W. J., Stone, B. L., Brown, B. A., Wallace, R. J. and Bottger, E. C. AIDS-related *Mycobacterium kansasii* infection with initial resistance to clarithromycin. Diagn. Microbiol. Infect. Dis. **31** (1998) 369–371.

Clarithromycin is a promising drug for the treatment of *Mycobacterium kansasii* infection. We report a patient with AIDS and severe *M. kansasii* infection who had previously received a short course of clarithromycin for sinusitis. He had clinical failure of treatment using clarithromycin plus ethambutol, and the initial isolate was found to be highly resistant to clarithromycin. Nucleotide sequencing of the 23S rRNA gene of this isolate demonstrated a single base mutation at position 2058, the same as that found in clarithromycin-resistant *M. avium*.—Authors' Abstract

de Soldenhoff, R., Hatta, M. and Weling Siro, T. Choosing the decolourizer and its strength to stain *Mycobacterium leprae*: does it actually matter? Lepr. Rev. **69** (1998) 128–133.

Leprosy bacilli are more easily decolorized during staining than tuberculosis bacilli, so a weaker concentration of decolorizer is usually recommended. In Indonesia, the same "strong" decolorizer is used for identifying both organisms. In a study to compare the results using different concentrations of different decolorizers, no difference could be found in the bacterial index (BI). It is suggested that the same staining technique can be used for tuberculosis and leprosy.—Authors' Summary

Dick, T., Lee, B. H. and Murugasu Oei, B. Oxygen depletion induced dormancy in *Mycobacterium smegmatis*. FEMS Microbiol. Lett. **163** (1998) 159–164.

We report here that the physiological behavior of the fast-growing saprophytic *Mycobacterium smegmatis* under *in vitro* oxygen-depletion and reactivation conditions is strikingly similar to the characteristics shown by the slow-growing pathogenic *M. tuberculosis*. *M. smegmatis* died rapidly when shifted abruptly from aerobic to anaerobic conditions. In contrast to the lethal shock of abrupt oxygen depletion, the slow depletion through a self-generated oxygen gradient permitted an adaptation to a persistent state which showed increased resistance against the bactericidal effects of anaerobiosis. The anaerobic persistent culture did not synthesize DNA and showed synchronized division upon reactivation in oxygen-rich medium, indicating that the persistent bacilli are uniformly arrested at a defined stage of the cell cycle. Upon reactivation the persistent culture started synthesizing DNA only after the first cell division, suggesting that the persistent cells contain two chromosomes. Furthermore, the persistent culture developed sensitivity to metronidazole and resistance against ofloxacin. These results suggest that *M. smegmatis* might be useful as a fast-growing non-pathogenic model for comparative molecular analyses of mycobacterial dormancy.—Authors' Abstract

Gomez, M., Doukhan, L., Nair, G. and Smith, I. *sigA* is an essential gene in *Mycobacterium smegmatis*. Mol. Microbiol. **29** (1998) 617–628.

sigA encodes a σ factor of the σ^{70} family, σ^{A}, that is found in all mycobacterial species. As σ^{A} shows high similarity to the primary σ factor in *Streptomyces coelicolor,* it was postulated that σ^{A} has the same role in mycobacteria. However, a point mutation in *sigA,* resulting in the replacement of arginine 522 by histidine, was found responsible for the attenuated virulence of the *Mycobacterium bovis* strain ATCC 35721. This raised the possibility that σ^{A} was an alternative σ factor specifically required for virulence gene expression. In this work, we show that *sigA* cannot be disrupted in *M. smegmatis* unless an extra copy of the gene is provided at another chromosomal site, which demonstrates that *sigA* is essential. To characterize the pattern of *sigA* expression during exponential and stationary phase in *M. smegmatis,* we measured the ß-galactosidase activity in a strain carrying a *sigA-lacZ* transcriptional fusion and monitored σ^{A} levels using Western blotting. Our results indicate that *sigA* is expressed throughout the growth of the culture. The essential character of *sigA* and its pattern of expression corroborate the hypothesis that *sigA* codes for the primary σ factor in *M. smegmatis* and, most likely, in all mycobacteria.—Authors' Summary

Lagier, B., Pelicic, V., Lecossier, D., Prod'hom, G., Rauzier, J., Guilhot, C., Gicquel, B. and Hance, A. J. Identification of genetic loci implicated in the survival of *Mycobacterium smegmatis* in human mononuclear phagocytes. Mol. Microbiol. **29** (1998) 465–475.

A luminescence-based procedure that permits the rapid evaluation of the survival of mycobacteria within mononuclear phagocytes was developed and used to screen insertional mutants of *Mycobacterium smegmatis* for their ability to survive in human monocyte-derived macrophages. Among the 5000 mutants tested, eight mutants were identified that demonstrated impaired intracellular survival in human macrophages but that grew normally in the absence of cells. For each mutant, a portion of the gene interrupted by the transposition event was amplified by ligand-mediated PCR and sequenced. In all cases, the existence of homologous genes of as yet unknown function were identified in the *M. tuberculosis* genome. Complementation of the mutant mycobacterial strains with cosmids containing the homologous loci from *M. tuberculosis* restored normal intracellular growth in three of the four mutants tested, supporting the idea that these loci contain genes that are important for intracellular survival. This study demonstrates the feasibility of directly screening mutant mycobacterial strains to identify genes coding for activities necessary for the intracellular survival in human mononuclear phagocytes, an important initial step in the identification of potential targets for new therapeutic agents.—Authors' Summary

Putra, S. R., Disch, A., Bravo, J. M. and Rohmer, M. Distribution of mevalonate and glyceraldehyde 3-phosphate/pyruvate routes for isoprenoid biosynthesis in some gram-negative bacteria and mycobacteria. FEMS Microbiol. Lett. **164** (1998) 169–175.

Labeling experiments using [1-C-13] acetate of [1-C-13] glucose were performed with opportunistic pathogenic bacteria, with innocuous bacteria related to pathogenic species or with phyropathogenic species. The labeling pattern was determined in the isoprenic moiety of ubiquinone or menaquinone derivatives. These experiments showed that *Acinetobacter, Citrobacter, Erwinia, Pseudomonas, Burkholderia, Ralstonia* and *Mycobacterium* synthesize their isoprenoids via the mevalonate-independent glyceraldehyde 3-phosphate/pyruvate route. Enzymes of this novel bacterial metabolic route, which is apparently absent in vertebrates and man, therefore represent potential targets for a novel type of antibacterial drugs.—Authors' Abstract

Rosenkrands, I., Rasmussen, P. B., Carnio, M., Jacobsen, S., Theisen, M. and Andersen, P. Identification and

characterization of a 29-kilodalton protein from *Mycobacterium tuberculosis* culture filtrate recognized by mouse memory effector cells. Infect. Immun. **66** (1998) 2728–2735.

Culture filtrate proteins from *Mycobacterium tuberculosis* induce protective immunity in various animal models of tuberculosis. Two molecular mass regions (6 to 10 kDa and 24 to 36 kDa) of short-term culture filtrate are preferentially recognized by Th1 cells in animal models as well as by patients with minimal disease. In the present study, the 24- to 36-kDa region has been studied, and the T-cell reactivity has been mapped in detail. Monoclonal antibodies were generated, and one monoclonal antibody, HYB 71-2, with reactivity against a 29-kDa antigen located in the highly reactive region below the antigen 85 complex was selected. The 29-kDa antigen (CFP29) was purified from *M. tuberculosis* short-term culture filtrate by thiophilic adsorption chromatography, anion-exchange chromatography, and gel filtration. In its native form, CFP29 forms a polymer with a high molecular mass. CFP29 was mapped in two-dimensional electrophoresis gels as three distinct spots just below the antigen 85 complex component MPT59. CFP29 is present in both culture filtrate and the membrane fraction from *M. tuberculosis,* suggesting that this antigen is released from the envelope to culture filtrate during growth. Determination of the N-terminal amino acid sequence allowed cloning and sequencing of the cfp29 gene. The nucleotide sequence showed 62% identity to the bacteriocin Linocin from *Brevibacterium linens.* Purified recombinant histidine-tagged CFP29 and native CFP29 had similar T-cell stimulatory properties, and they both elicited the release of high levels of gamma interferon from mouse memory effector cells isolated during the recall of protective immunity to tuberculosis. Interspecies analysis by immunoblotting and PCR demonstrated that CFP29 is widely distributed in mycobacterial species.—Authors' Abstract

Vasanthakrishna, M., Rumpal, N. and Varshney, U. Organization and copy number initiator tRNA genes in slow- and fast-growing mycobacteria. J. Biosci. **23** (1998) 101–110.

We have previously reported the isolation and characterization of a functional initiator tRNA gene, *metA,* and a second initiator tRNA-like sequence, *metB,* from *Mycobacterium tuberculosis.* Here we describe the fine mapping of the initiator tRNA gene locus of the avirulent (H37Ra) and virulent (H37Rv) strains of *M. tuberculosis.* The genomic blot analyses show that the 1.7 kb (harboring *metB*) and the 6.0 kb BamHI (harboring *metA*) fragments are linked. Further, sequencing of a portion of the 6.0 kb fragment, in conjunction with the sequence of the 1.7 kb fragment, confirmed the presence of an IS6110 element in the vicinity of *metB.* The IS element is flanked by inverted (28 bp, with 3 contiguous mismatches in the middle) and direct (3 bp) repeats considered to be the hallmarks of IS6110 integration sites. The organization of the initiator tRNA gene locus is identical in both the H37Ra and H37Rv strains and they carry a single copy of the functional initiator tRNA gene. Interestingly, the fast-growing *M. smegmatis* also bears a single initiator tRNA gene. This finding is significant in view of the qualitative differences in total tRNA pools and the copy number of rRNA genes in the fast- and slow-growing mycobacteria. Finally, we discuss hypotheses related to the origin of *metB* in *M. tuberculosis.*—Authors' Abstract

Weng, X., Qian, L., Zhu, K., Li, H. and Douglas, J. T. [Detection of *M. leprae* and PGL-I antigen in nasal secretion and skin tissue.] J. Clin. Dermatol. **27** (1998) 150–153. (in Chinese)

In order to better understand the role of nasal carriage of *Mycobacterium leprae* in the transmission of leprosy and to evaluate the efficacy in leprosy control with detection of *M. leprae* in nasal secretion, PCR and dot-ELISA/ECL were applied to detect *M. leprae* and PGL-I antigen in nasal secretion and skin tissue samples from 32 active leprosy patients, 13 cured patients and 143 household contacts. The comparative study

showed that dot-ELISA/ECL is a sensitive, specific, simple and rapid method; it can be used in the epidemiologic study on leprosy. In addition, the GVHP membrane is a good absorbent carrier suitable for the detection of mucosa-secreted antigen.—Authors' English Abstract

Yin, Y., *et al.* [Establishment of high-potent genome DNA library of *M. leprae* and its evaluation.] China Lepr. J. **14** (1998) 75–78. (in Chinese)

A genomic library of *Mycobacterium leprae* has been constructed by using λgtll as vector. The DNA was extracted from *M. leprae* harvested from nude mice infected with *M. leprae* Thai 53 strain. The plaque forming unit (pfu) of the genomic library is 6×10^4 pfu/ml and has been expanded to 2.6×10^8 pfu/ml as a permanent source of genomic library of *M. leprae.*—Authors' English Abstract

Yin, Y., *et al.* [On method of large preparation and purification of recombinant α-protein antigen.] China Lepr. J. **14** (1998) 79–81. (in Chinese)

Recombinant α1 and α2 antigens of *Mycobacterium leprae* have been constructed. The recombinant α antigen was efficiently expressed on *E. coli* by using pMALc-RI as an expression vector, and purified by amylose resin affinity chromatography. More than 6 mg and 10 mg recombinant α1 and α2 proteins were obtained from 200 ml liquid culture, respectively.—Authors' English Abstract

Zhu, W., Arceneaux, J. E. L., Beggs, M. L., Beyers, B. R., Eisenach, K. D. and Ludrigan, M. D. Exochelin genes in *Mycobacterium smegmatis*: identification of an ABC transporter and two non-ribosomal peptide synthetase genes. Mol. Microbiol. **29** (1998) 629–639.

Many strains of mycobacteria produce two ferric chelating substances that are termed exochelin (an excreted product) and mycobactin (a cell-associated product). These agents may function as iron acquisition siderophores. To examine the genetics of the iron acquisition system in mycobacteria, ultraviolet (UV) and transposon (Tn*611*) mutagenesis techniques were used to generate exochelin-deficient mutants of *Mycobacterium smegmatis* strains ATCC 607 and LR222, respectively. Mutants were identified on CAS siderophore detection agar plates. Comparisons of the amounts of CAS-reactive material excreted by the possible mutant strains with that of the wild-type strains verified that seven UV mutant strains and two confirmed transposition mutant strains were deficient in exochelin production. Cell-associated mycobactin production in the mutants appeared to be normal. From the two transposon mutants, the mutated gene regions were cloned and identified by colony hybridization with an IS*6100* probe, and the DNA regions flanking the transposon insertion sites were then used as probes to clone the wild-type loci from *M. smegmatis* LR222 genomic DNA. Complementation assays showed that an 8 kb *Pst*I fragment and a 4.8 kb *Pst*I/*Sac*I subclone of this fragment complemented one transposon mutant (LUN2) and one UV mutant (R92). A 10.1 kb *Sac*I fragment restored exochelin production to the other transposon mutant (LUN1). The nucleotide sequence of the 15.3 kb DNA region that spanned the two transposon insertion sites overlapped the 5′ region of the previously reported exochelin biosynthetic gene *fxbA* and contained three open reading frames that were transcribed in the opposite orientation to *fxbA*. The corresponding genes were designated *exiT, fxbB* and *fxbC*. The deduced amino-acid sequence of ExiT suggested that it was a member of the ABC transporter superfamily, while FxbB and FxbC displayed significant homology with many enzymes (including pristinamycin I synthetase) that catalyze non-ribosomal peptide synthesis. We propose that the peptide backbone of the siderophore exochelin is synthesized in part by enzymes resembling non-ribosomal peptide synthetases and that the ABC transporter ExiT is responsible for exochelin excretion.—Authors' Summary

Epidemiology and Prevention

Cree, I. A. and Smith, W. C. Leprosy transmission and mucosal immunity: towards eradication? Lepr. Rev. **69** (1998) 112–121.

The declining prevalence of leprosy has not been matched by a declining incidence. Widespread adoption of multiple drug therapy (MDT) in closely monitored control programs has not prevented transmission of *Mycobacterium leprae*. Despite the rarity of lepromatous patients, most of those living in endemic areas have immunological evidence of exposure to *M. leprae*. This paradox could be explained if, for many such individuals, infections was transient, did not result in disease development, but did allow the transmission of infection to other individuals. There is increasing evidence from nasal PCR studies that such subclinical transmission may exist and that mucosal immune responses to *M. leprae* may develop during resolution of initial infection. Subclinical infection appears to occur in clusters and may require close contact over a prolonged period for optimal transmission. Control of transmission may be feasible through identification and treatment of individuals within infection clusters, allowing progress toward the eradication of leprosy.—Authors' Summary

Gil Suarez, R. E. and Lombardi, C. [Estimated prevalence of leprosy.] Hansenol. Int. **22** (1997) 31–34. (in Spanish)

According to data of WHO, it is considered that a hidden prevalence exists in a number of 250,000 cases, approximately, distributed in the major endemic countries. This situation is the product of the characteristics of the illness and operational factors that hinder the early diagnosis.

On the other hand, the programs of control need to have estimated data about this hidden prevalence in order to plan their actions and to evaluate their results, for what was elaborated a simple method that allows to carry out this estimate. Although it is recognized that the confidence of the method depends on the quality of the data used in the procedure, it only allows to come closer to reality, without being exactly this reality.

The method is based on the fact, generally accepted, that the patients diagnosed early must present less disabilities, for what was considered that the percent of new cases diagnosed with some disability represents an indicator for the average of the incubation period. This estimate should involved the 5 years previous to the year in which we seek to estimate the hidden prevalence.

The real estimated prevalence would be the results of the estimated hidden prevalence plus the well-known prevalence. To evaluate the results of the search actions of this serious hidden prevalence it is expected that the new cases detected in this particular year were similar to the sum of the estimated hidden prevalence added of the average of the new detected cases of routine in the last 5 years.—Authors' English Summary

Gupte, M. D., Vallishayee, R. S., Masood Ahmed, T. H., Prince, J. S., De Britto, R. L. J., Rathinaraj, B., Elango, N., Balasubramanyam, S., Nagaraju, B. and Arockiasamy, J. Studies on rapid assessment methods in leprosy. Indian J. Lepr. **70** (1988) 165–177.

A study was undertaken in Pudukottai district, Tamil Nadu, India, to test rapid assessment methods; viz (i) sample surveys with lower coverages for clinical examination in estimating the disease problem in the community, (ii) utility of registered case prevalence for estimating the actual prevalence in a given area, (iii) leprosy in school-going children and its utility in estimating leprosy prevalence in the community, and (iv) information on disability and smear positivity in estimating leprosy prevalence; and develop correction factors for estimating leprosy situation.

A sample of 23 clusters from 582 clusters of contiguous villages and hamlets was further divided into two random sub-samples for two surveys with differing coverages.

One team covered nine clusters comprising 34 villages with a population of 17,562 and examined 15,596 (89%) persons for leprosy. A second team covered 14 clusters comprising 86 villages with a population of 26,927 and examined 16,622 (62%) persons for leprosy.

The results showed that: (i) leprosy sample surveys with lowered coverages would tend to miss valuable information, in terms of quality and quantity; (ii) information from "known case" registers to estimate the true burden of leprosy disease and to monitor its trend over time is inadequate; (iii) school surveys are of limited value for estimating the disease burden in the community or to monitor its trend over time; (iv) the number of smear-positive cases is too small to serve as an indicator for the total case load in the community; and (v) the prevalence of active disease and that of grade 2 disability in the community are poorly correlated.

Reliable methods other than those used here need to be developed for evaluation and monitoring of the disease burden, particularly in the post-MDT era.—Authors' Abstract

Ladhani, S. Leprosy in Pakistan: LEPRA Elective Study. Lepr. Rev. **69** (1998) 164–167.

As part of the curriculum, medical students at the United Medical and Dental School of Guy's and St. Thomas's Hospitals (UMDS), London, are encouraged to spend an elective period of 8 weeks in their final year anywhere in the world, studying any field of medicine they are interested in. Having lived in Tanzania for 10 years, I have had contact with people suffering from leprosy and my interest in leprosy continued after I moved to Europe to continue my education. I therefore decided to use my elective to gain hands-on experience with the disease so that I could understand and appreciate the impact of leprosy in developing countries such as Pakistan.—Author's Summary

Niu, S., *et al.* [Leprosy and climate.] China Lepr. J. **14** (1998) 101–103. (in Chinese)

There is a close relationship between leprosy incidence and the climate, based on the analysis of the distribution maps of leprosy in Anhui and China. The authors emphasized that leprosy can be controlled through improving the micro-climate (biological environment) and putting on shoes to cultivate and to walk.—Authors' English Abstract

Smith, C. Leprosy in the balance—predicting the future. Nu Nytt om U-Landshalsovard **11** (1997) 35–36.

This paper addresses the topic of what will happen to leprosy after the year 2000. It considers the current trends in leprosy as a balance between the host, the agent and the environment, identifies the factors which may influence future trends, and presents an analysis of possible scenarios.—Trop. Dis. Bull. **95** (1998) 733

Song, X., *et al.* [Analysis of 87 newly detected cases of leprosy for the past 12 years.] China Lepr. J. **14** (1998) 94. (in Chinese)

In Linyi City, Shandong, China, with a population of 9,700,000, leprosy prevalence of 1.27‰ has reduced to less than 0.01‰ in 1989. From 1985 to 1996 there have been 87 new leprosy patients detected, being 3 to 11 or an average of 7.25 cases yearly, including LL 27, B 34 and TT 26, 70 male and 17 female with average age of 47 (22 to 72) years and disease duration of 6 months to 32 years. Among them 78 (89.7%) and 9 (10.3%) cases were misdiagnosed in provincial or municipal hospitals and even in special leprosy control units, respectively. There was one case with a wrong diagnosis of rheumatism who had been treated with prednisone for as long as 4 years. The authors emphasized that the drive for leprosy control should never be slackened after the goal of basically eradicating it has been reached, and that the most important is popularization of knowledge of diagnosing leprosy in general medical workers and even in leprosy control units.—Authors' English Abstract

Tiendrebeogo, A., Sow, S. O., Sawadogo, O., Dembele, M. S., Ouedraogo, K., Bide, L. and Millan, J. [Evaluation of the elimination of leprosy in Burkina Faso.] Acta Leprol. **11** (1998) 7–16. (in French)

During May and June 1997, we conducted a rapid survey on leprosy prevalence in 30 villages. It was to assess reaching of the leprosy elimination threshold (1 case per 10,000 inhabitants) in Burkina Faso. We drew lots for the villages in 10 provinces among which 5 had the highest prevalence rates of leprosy in 1996 and 5 had the lowest prevalence rates. We added a leprosy elimination monitoring to the survey. This monitoring consisted of visits to the health centers covering the 30 villages. We interviewed and clinically examined 33 cases of leprosy in treatment in those health centers. We found 51 patient of leprosy in the visited villages. The prevalence rate of leprosy (6.74 per 10,000 inhabitants) was twice higher than the prevalence rate registered in the same villages. We detected 28 new cases of leprosy during the survey. Proportion of hidden cases of leprosy were 54.9%. We estimated geographical coverage of MDT at 75% in the 10 provinces. Eight of the 27 visited health centers (29.6%) did not get sufficient supplies. The cure rate has fallen from 93% to 73% between 1992 and 1997. Our results show that leprosy elimination threshold is not reached in Burkina Faso. Leprosy control activities that were declining during the last 5 years need to be reinforced.—Authors' English Summary

Vijayakumaran, P., Reddy, N. B. B., Krishnamurthy, P. and Ramanujam, R. NLEP; utilizing primary health care workers for case detection. Indian J. Lepr. **70** (1998) 203–210.

Under the [Indian] National Leprosy Elimination Programme (NLEP) it takes at least 1 year for the paramedical worker to survey the allotted population for case detection. An alternative strategy is warranted for states like Bihar still having a high case load and poorly functioning leprosy program. An intensive case-finding program using primary health care (PHC) workers was organized in Bhojpur district, Bihar, India. The whole population (3,173,701 in 1996) of the district was screened within a period of 4 days and confirmation of suspected cases was carried out in 4 days. During this screening procedure, 1586 new leprosy cases were detected (NCDR = 5 cases per 10,000) and all were started on MDT. The new cases constituted 26.4% of active cases existing on record before the screening. After this experience, the prevalence rate of active cases increased from 19 to 24 per 10,000. If such rapid screening programs are done at least twice a year, it will greatly hasten the process of elimination of leprosy.—Authors' Abstract

World Health Organization. Global case-detection trend in leprosy. Wkly. Epidemiol. Rec. **72** (1997) 173–180.

This report analyzes trends in leprosy over 12 years (1985–1996) in the top 28 endemic countries (which represent 95% of the current worldwide leprosy burden and 80% of the leprosy burden as it was in 1985), and discusses the extent to which changes in the detection and in the profile of newly detected cases reflect changes in the transmission of the disease.—Trop. Dis. Bull. **95** (1998) 733

World Health Organization. Leprosy in the balance—predicting the future. Nu Nytt om U-Landshalsovard **11** (1997) 9–10, 35–36.

Three maps are presented illustrating the progress made toward global leprosy elimination up to 1997. The first shows countries which have eliminated the disease as a public health problem over the last 10 years and countries that are still endemic; the second map shows the prevalence of leprosy in the world at the beginning of 1997 per 10,000 population; the final map gives the detection rates for leprosy per 100,000 population in 1996. The distribution of leprosy remains very uneven between countries; however the disease is more prevalent in regions situated in the inter-tropical belt. Transmission of the disease is still significant in Africa, Latin America, and South-East Asia.—Trop. Dis. Bull. **95** (1998) 733

Rehabilitation

Carpintero, P., Logrono, C., Carreto, A., Carrascal, A. and Lluch, C. Progression of bone lesions in cured leprosy patients. Acta Leprol. **11** (1998) 21–24.

A group of 52 patients deemed to be cured of Hansen's disease were examined in order to determine the appearance or aggravation of bone lesions after cure. A study was made of X-rays performed both at the moment these patients were considered to be cured and a minimum of 2 years later. During the elapsing interval, new lesions had appeared in 8 patients, and existing lesions had worsened in 12 patients. Factors associated with the progression of lesions were: impaired sensitivity, physical activity and appearance of plantar ulcers. The authors feel that leprosy patients, even when considered to be bacteriologically cured, should undergo regular checkups. Factors which might aggravate bone lesions should be borne in mind.—Authors' Abstract

Huang, S., *et al.* [Treatment of ulcers on the heel with plantar aponeurosis—skin flap in leprosy.] China Lepr. J. **14** (1998) 95. (in Chinese)

A plantar aponeurosis flap was used for healing plantar ulcers on the heel of five persons cured of leprosy. These ulcers were persisting for over 10 years. To take such a flap did not cause obvious defect in its supply area or appearance or function of the foot. The reconstructed heel is similar to a normal one.—Authors' English Abstract

Husain, S., Mishra, B., Prakash, V. and Malaviya, G. N. Results of surgical decompression of ulnar nerve in leprosy. Acta Leprol. **11** (1998) 17–20.

Ulnar neurolysis in 279 cases of leprosy was performed with the objectives of relief in neuritic pain and impending/existing sensory motor loss of varying extent. Of the above, 193 could be followed between 3–10 years. Neuritic pain was first to disappear; 48.7% of the 193 cases showed sensory recovery. Motor power gain and/or further fall in muscle power was prevented as a result of preventive neurolysis in 173 (89.6%) cases. In this series, benefits of appropriate and timely surgical intervention have been observed.—Authors' Summary

Kumaravel, S. Neoplastic transformation of chronic ulcers in leprosy patients—a retrospective study of 12 consecutive cases. Indian J. Lepr. **70** (1998) 179–187.

A retrospective analysis of chronic ulcers among leprosy patients seen over the last 20 years yielded 23 cases of neoplastic transformation. It showed a peak at the sixth decade, an incidence of 3.66/100 among hospitalized ulcer cases and a male/female ratio of 1.6:1. Borderline tuberculoid was the most common type of leprosy involved (40%). Squamous cell carcinoma was the most common neoplasia. Its usual site was plantar ulcers. Heel ulcers showed relatively greater predilection for malignancy (38.5%). Histopathological proof of malignancy is desirable and that may require multiple biopsies. Metastasis is rare but potentially fatal. The surgical treatment must provide a functional, trouble-free limb. Forefoot or Lisfranc's amputation for distal third ulcers and below-knee amputation for large midfoot and heel ulcers are procedures of choice. Wide excision may be used in selected cases.—Author's Abstract

Mao, Q., *et al.* [Comprehensive treatment of 108 complicated plantar ulcers in leprosy.] China Lepr. J. **14** (1998) 91. (in Chinese)

Since September 1995, 108 leprosy patients with long-term and complicated plantar ulcers, whose mean age was 61.7 years, have accepted a comprehensive therapy consisting of sequestrectomy, debridement, regular dressing and infrared radiation and bed rest. The results showed that 60 of 64 inpatients (93.8%) and 13 of 44 outpatients (29.6%) have been healed.—Authors' English Abstract

Wang, Q., *et al.* [Efficacy of 18-month self-care in leprosy.] China Lepr. J. **14** (1998) 90–91. (in Chinese)

From June 1995 to December 1996, 250 persons cured of leprosy (including male 184 and female 66) with a mean age of 47.4 years, of whom 240 had grade II (WHO) disability, have been taught to do self-care for their eyes, hands and feet and evaluated regularly. After 18 months, pink eyes decreased from 50 to 35, chaps in hands and feet from 26 to 8, and plantar ulcers from 77 to 65. The authors pointed out that if the self-care is upheld for a long time, rehabilitation to some extent will indeed be promoted.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Adjei, O., Evans, M. R. W. and Asiedu, A. Phenytoin in the treatment of Buruli ulcer. Trans. R. Soc. Trop. Med. Hyg. **92** (1998) 108–109.

Three cases of Buruli ulcer (caused by *Mycobacterium ulcerans*) in Ghana which were successfully treated by the topical application of phenytoin powder are described.—Authors' Abstract

Argiles, J. M., Carbo, N. and Lopez Soriano, F. J. Was tumour necrosis factor-alpha responsible for the fetal malformations associated with thalidomide in the early 1960s? Med. Hypotheses **50** (1998) 313–318.

Prescription of thalidomide as a sedative to pregnant women in the early 1960s resulted in a dramatic number of fetal malformations that affected over ten thousand babies. Although tumor necrosis factor-alpha (TNF-α) is basically a cytotoxic molecule produced by macrophages when activated by invasive stimuli (such as bacterial endotoxin or tumor growth), it could have an important role in pregnancy, especially in early embryonic development. On these lines, both in human subjects and experimental animals, the cytokine is expressed and synthesized in endometrium, placenta and fetus. Evidence is presented here suggesting that the embryonic action of thalidomide was mediated by TNF-α, since the drug is a powerful inhibitor of the synthesis of this cytokine.—Authors' Abstract

Bartow, R. A. and McMurray, D. N. Lymphocytes expressing Fc gamma receptors suppress antigen-induced proliferation in cells from guinea pigs infected with *Mycobacterium tuberculosis*. Cell. Immun. **184** (1998) 51–57.

The immunomodulatory role of T lymphocytes expressing receptors for the Fc portion of IgG (Ty cells) in BCG-vaccinated guinea pigs following pulmonary infection with a low dose of virulent *Mycobacterium tuberculosis* H37Rv was studied. Compared to uninfected animals, guinea pigs infected 2 or 4 weeks previously harbored significantly increased percentages of Ty cells in the peripheral blood (twofold increase) and the spleen (50% increase), and at 4 weeks had nearly fourfold increases in Ty cells in bronchotracheal lymph nodes draining the infected lungs. Removal of Ty cells by panning on plastic dishes coated with a monoclonal antibody specific for guinea pig Fc gamma R resulted in significant increases in proliferative responses of splenocytes to ConA and PPD *in vitro*. Removal of Ty cells from peripheral blood lymphocytes resulted in significantly increased responses to PPD and to recombinant mycobacterial hsp 65 and hsp 70 antigens. The isolated Ty cells themselves did not proliferate when stimulated with ConA, PPD, or either of the specific mycobacterial antigens, even in the presence of syngeneic accessory cells. These results suggest that FcR gamma-bearing T cells may play an important immunomodulatory role in pulmonary tuberculosis, principally by sup-

pressing antigen-induced proliferation in the rest of the lymphocyte population.—Authors' Abstract

Bermudez, L. E., Petrofsky, M. and Stevens, P. Treatment with recombinant granulocyte colony-stimulating factor (Filgrastin™) stimulates neutrophils and tissue macrophages and induced an effective nonspecific response against *Mycobacterium avium* in mice. Immunology **94** (1998) 297–303.

A role of neutrophils in the host response against *Mycobacterium avium* (MAC) has recently been suggested. To investigate this matter further, we determined the effect of granulocyte colony-stimulating factor (G-CSF) on the outcome of MAC infection in mice. C57BL/6 bg(+)/bg(−) black mice were intravenously infected with 1×10^7 MAC and then divided into four experimental groups to receive G-CSF as follows: (i) 10 μg/kg/day; (ii) 50 μg/kg/day; (iii) 100 μg/kg/day; (iv) placebo control. Mice were killed at 2 and 4 weeks of treatment to determine the bacterial load of liver and spleen. Treatment with G-CSF at both 10 and 50 μg/kg/day doses significantly decreased the number of viable bacteria in liver and spleen after 2 weeks (approximate to 70.5% and 69.0%, respectively), and after 4 weeks (approximate to 53% and 52%, respectively, $p < 0.05$ compared with placebo control). Treatment with 100 μg/kg/day did not result in decrease of bacterial colony-forming units in the liver and spleen after 4 weeks. Administration of G-CSF induced interleukin-10 (IL-10) and IL-12 production by splenocytes. To examine if the protective effect of G-CSF was accompanied by the activation of phagocytic cells, blood neutrophils and splenic macrophages were purified from mice receiving G-CSF and their ability to kill MAC was examined *ex vivo*. Neutrophils and macrophages from G-CSF-treated mice were able to inhibit the growth of or to kill MAC *ex vivo,* while phagocytic cells from untreated control mice had no anti-MAC effect. These results suggest that activation of neutrophils appears to induce an effective nonspecific host defense against MAC, and further studies should aim for better understanding of the mechanisms of protection.—Authors' Abstract

Cantoni, R., Branzoni, M., Labo, M., Rizzi, M. and Riccardi, G. The MTCY428.08 gene of *Mycobacterium tuberculosis* codes for NAD^+ synthetase. J. Bacteriol. **180** (1998) 3218–3221.

The product of the MTCY428.08 gene of *Mycobacterium tuberculosis* shows sequence homology with several NAD^+ synthetases. The MTCY428.08 gene was cloned into the expression vectors pGEX-4T-1 and pET-15b. Expression in *Escherichia coli* led to overproduction of glutathione S-transferase fused and His^6-tagged gene products, which were enzymatically assayed for NAD synthetase activity. Our results demonstrate that the MTCP428.08 gene of *M. tuberculosis* is the structural gene for NAD^+ synthetase.—Authors' Abstract

Choonhakaran, C., Chetchotisakd, P., Jirarattanapochai, K. and Mootsikapun, P. Sweet's syndrome associated with nontuberculous mycobacterial infection: a report of five cases. Br. J. Dermatol. **139** (1998) 107–110.

We report the rare association of Sweet's syndrome with nontuberculous mycobacteria in five patients (three women, two men, aged 25–41 years). Clinical and histological evidence supported the diagnosis of Sweet's syndrome in all patients. The skin lesions responded well to systemic corticosteroid but recurred in two cases. All of our patients had chronic disseminated nontuberculous mycobacterial infection. They initially presented with lymphadenopathy and developed involvement in other organs later. All of them were treated as having tuberculous lymphadenitis based on pathological findings before definite diagnosis was made by culture. The organisms isolated were *Mycobacterium chelonae* in three cases, *M. scrofulaceum* in one case and *M. avium intracellulare* complex in one case. All the patients gradually improved with treatment but one had multiple recurrences. The search for an infectious agent, especially nontuberculous mycobacteria,

should be performed in cases of Sweet's syndrome that appear in association with chronic granulomatous lymphadenitis which is recalcitrant to antituberculous drugs.—Authors' Abstract

Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S., Barry, C. E., Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., *et al.* Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393** (1998) 537.

Countless millions of people have died from tuberculosis, a chronic infectious disease caused by the tubercle bacillus. The complete genome sequence of the best-characterized strain of *Mycobacterium tuberculosis,* H37Rv, has been determined and analyzed in order to improve our understanding of the biology of this slow-growing pathogen and to help the conception of new prophylactic and therapeutic interventions. The genome comprises 4,411,529 base pairs, contains around 4000 genes, and has a very high guanine + cytosine content that is reflected in the biased amino-acid content of the proteins. *M. tuberculosis* differs radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved in lipogenesis and lipolysis, and to two new families of glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation.—Authors' Abstract

DasGupta, S. K., Jain, S., Kaushal, D. and Tyagi, A. K. Expression systems for study of mycobacterial gene regulation and development of recombinant BCG vaccines. Biochem. Biophys. Res. Commun. **246** (1998) 797–804.

Successful genetic engineering of mycobacteria is crucial for developing new approaches to combat tuberculosis as well as for dissecting out the molecular basis of pathogenesis of *Mycobacterium tuberculosis.* We have constructed a *Mycobacterium-Escherichia coli* shuttle expression vector pSD5. It carries a modular expression cassette which provides sites for cloning of promoters, a ribosome binding site (RBS) with an appropriately placed initiation codon and multiple cloning sites for cloning the genes of interest. We also constructed pDK20, an integration proficient derivative of pSD5, by incorporating mycobacteriophage L5 integration signals in lieu of the origin of DNA replication for mycobacteria. This vector permits stable expression of genes in *M. bovis* BCC, *M. smegmatis,* and *M. tuberculosis* under the transcriptional control of a mycobacterial promoter. These vectors enable the expression of a gene to be regulated by several hundred-fold depending upon the strength of mycobacterial promotor. We propose that expression of protective antigens using an appropriate promoter derivative of pDK20 should help in development of recombinant BCG vaccines that can induce an optimal immune response from the host. We have further employed the integration proficient expression system for comparing the efficiency and specificity of transcriptional recognition in *M. bovis* BCG, *M. tuberculosis,* and *M. smegmatis.* We show that fast-growing *M. smegmatis* and slow-growing *M. tuberculosis* and *M. bovis* BCG recognize mycobacterial promoters with comparable efficiency in spite of differences in their growth rates.—Authors' Abstract

Desjardin, L. E., Chen, Y., Perkins, M. D., Teixeira, L., Cave, M. D. and Eisenach, K. D. Comparison of the ABI 7700 system (TaqMan) and competitive PCR for quantification of IS6110 DNA in sputum during treatment of tuberculosis. J. Clin. Microbiol. **36** (1998) 1964–1968.

Mycobacterium tuberculosis can persist in sputum for long periods of time after the initiation of antituberculosis chemotherapy. The purpose of this study was to determine whether quantitative estimates of *M. tuberculosis* DNA in sputum correlate with the numbers of viable bacilli and thus measure the therapeutic response of patients during treatment. Two methods of *M. tuberculosis* DNA quantification were examined by using DNA isolated from sputum specimens

serially collected during the course of chemotherapy. A competitive PCR assay was compared to an automated system of real-time quantification with the ABI Prism 7700 Sequence Detection System (TaqMan). The ABI 7700 system uses standard PCR in conjunction with a fluorogenic probe in which the intensity of fluorescence is proportional to the amount of target DNA present. The results showed that both PCR systems are reproducible and accurate. The amounts of *M. tuberculosis* DNA quantified in sputum corresponded well with the numbers of acid-fast bacilli (AFB) counted by microscopy. Before initiation of antituberculosis therapy, measures of AFB, *M. tuberculosis* DNA, and cultivable bacilli were similar, suggesting that quantification of DNA is a good method for measuring the initial bacillary load. However, the rate of disappearance of both AFB and *M. tuberculosis* DNA did not correlate with the decline in cultivable bacilli in the specimen; therefore, these tests are not appropriate for monitoring treatment efficacy.—Authors' Abstract

Gamboa, F., Cardona, P. J., Manterola, J. M., Lonca, J., Matas, L., Padilla, E., Manzano, J. R. and Ausina, V. Evaluation of a commercial probe assay for detection of rifampin resistance in *Mycobacterium tuberculosis* directly from respiratory and nonrespiratory clinical samples. Eur. J. Clin. Microbiol. Infect. Dis. **17** (1998) 189–192.

A commercial assay (Inno-Line Probe Assay; Innogenetics, Belgium) was evaluated to determine its ability to detect rifampin resistance in *Mycobacterium tuberculosis* directly from clinical specimens. Fifty-nine selected specimens (42 respiratory and 17 nonrespiratory) culture-positive for *M. tuberculosis* were tested along with their corresponding isolates in culture. The results were compared with those obtained by *in vitro* susceptibility testing. The results of the line probe assay to detect rifampin resistance in *M. tuberculosis* present in clinical specimens and in cultured isolated were concordant for 58 of 59 (98.3%) isolates (95% confidence limits = 90.9%–99.9%). The line probe assay failed only once, when a fecal specimen was tested: no amplification was observed due to the presence of inhibitory compounds. The most frequently observed mutation was His(526) → ASP (58.7%), followed by the His(526) → Tyr (23.9%); together, they represented 82.6% of rifampin-resistant samples. In conclusion, the Inno-Line Probe Assay is a rapid, useful method for detecting the presence of *M. tuberculosis* complex and its resistance to rifampin directly from clinical specimens and culture. Moreover, since rifampin resistance is a potential marker for multidrug resistance in *M. tuberculosis*, this assay may constitute an important tool for the control of tuberculosis.—Authors' Abstract

Gladwin, M. T., Plorde, J. J. and Martin, T. R. Clinical application of the *Mycobacterium tuberculosis* direct test: case report, literature review, and proposed clinical algorithm. Chest **114** (1998) 317–323.

The relatively new *Mycobacterium tuberculosis* direct test (MTDT) enzymatically amplifies *M. tuberculosis* complex 16s ribosomal RNA. The sensitivity of the test ranges from 75% to 100%, with specificity of 95% to 100%, positive predictive value between 78% and 100%, and negative predictive value between 95% and 100%. Similar test characteristics have been documented in nonrespiratory specimens and in specimens that ultimately grow nontuberculous mycobacterium (NTM). This test allows for rapid identification of *M. tuberculosis* in the smear-positive patient and may greatly improve sensitivity over acid-fast bacilli smear alone. A negative test result with a positive smear suggests infection with NTM or *M. avium* complex. We present a case that illustrates the value of MTDT for analysis of tissue specimens in immunocompromised patients with suspected mycobacterial disease and review the rapidly developing literature about this test. We propose an algorithm using MTDT, acid-fast smear, and mycobacterial culture for the diagnosis and treatment of the immunocompromised patient with suspected mycobacterial infection.—Authors' Abstract

Hashimoto, Y. Novel biological response modifiers derived from thalidomide. Curr. Med. Chem. **5** (1998) 163–178.

Thalidomide (N-alpha-phthalimidoglutarmimide) was used widely as a hypnotic/sedative agent in the late 1950s and the early 1960s, but had to be withdrawn from the market because of its severe teratogencity. In spite of this, there has been a resurgence of interest in the drug in recent years due to its potential usefulness for the treatment of various diseases, including acquired immunodeficiency syndrome (AIDS) and graft-versus-host disease (GVHD). The effectiveness of the drug in these diseases has been attributed to its specific inhibitory activity on tumor necrosis factor-alpha (TNF-α) production. Because TNF-α, a cytokine-mediating host defense and immune regulation, with a wide range of activities, has deleterious pathophysiological effects in various diseases, including AIDS, tumors, rheumatoid arthritis and diabetes, its production-regulators are attractive lead compounds for novel biological response modifiers. The regulatory effect of thalidomide on TNF-α production has been found to be bidirectional, depending on both the cell-type and the TNF-α production-inducer; i.e., thalidomide possesses both enhancing and inhibiting activities on TNF-α production. Structural modification of thalidomide aiming at the creation of superior TNF-α production-regulators has afforded a number of phenyl- and benzylphthalimide analogs possessing more potent activity than thalidomide itself. The structure-activity relationships of these analogs has been investigated. The bidirectional TNF-α production-regulating activity is electronic state- and enantio-dependent, and both pure inhibitors and pure enhancers of TNF-α production has been obtained. Further structural development of the phthalimide analogs has yielded potent nonsteroidal androgen antagonists.—Author's Abstract

Hoft, D. F., Brown, R. M. and Roodman, S. T. Bacille Calmette-Guerin vaccination enhances human gamma delta T cell responsiveness to mycobacteria suggestive of a memory-like phenotype. J. Immunol. **161** (1998) 1045–1054.

Bacille Calmette-Guerin (BCG) immunity can be studied as one experimental model for mycobacterial protective immunity. We have used flow cytometry to investigate human T-cell subsets induced by BCG vaccination. PBMC harvested from BCG-vaccinated individuals and controls were stimulated with mycobacterial Ags, and the T-cell subsets present after 7 days of *in vitro* expansion were characterized. The most dramatic expansions induced by mycobacterial Ags were detected in gamma delta T cells. The gamma delta T-cell expansions measured after *in vitro* stimulation with mycobacterial Ags were significantly greater in BCG responders compared with nonsensitized controls, indicating that BCG vaccination induced gamma delta T-cell activation associated with enhanced secondary responses. The majority of gamma delta T cells induced by BCG vaccination were gamma(9)(+)delta(2)(+) T cells reactive with isophenyl pyrophosphates. Coculture with CD4(+) T cells induced optimal gamma delta T-cell expansion, although IL-2 alone could provide this helper function in the absence of CD4(+) T cells. Gamma delta T cells were found to provide helper functions for mycobacterial specific CD4(+) and CD8(+) T cells as well, demonstrating reciprocal stimulatory interactions between gamma delta T cells and other T-cell subsets. Finally, prominent mycobacterial specific gamma delta T-cell expansions were detected in a subset of unvaccinated controls with evidence for prior sensitization to mycobacterial lysates (elevated mycobacterial specific lympho-proliferative responses). These latter findings are consistent with the hypothesis that exposure to atypical mycobacteria or related environmental Ags may induce gamma delta T cells crossreactive with Ags present in the *Mycobacterium tuberculosis* complex. Our results suggest that gamma delta T cells may be capable of developing a memory immune-like phenotype, and therefore might be important targets for new vaccines.—Authors' Abstract

Kamat, S. R., Dawson, J. J. Y., Devadatta, S., Fox, W., Janardhanam, B., Radhakrishna, S., Ramakrishnan, C. V., Somasundaram, P. R., Stott, H.

and Velu, S. A controlled study of the influence of segregation of tuberculous patients for 1 year on the attack rate of tuberculosis in a 5-year period in close family contacts in South India. [Reprinted from Bull. WHO **34** (1966) 517–532]—Bull. WHO **76** (1998) 109–124.

This report is the last of a series of nine publications from the Tuberculosis Chemotherapy Centre, Madras, India, concerning various aspects of an investigation of the role of ambulatory chemotherapy for pulmonary tuberculosis. It presents the attack rates of tuberculosis over a 5-year period of follow up of close family contacts of patients, all of whom were treated for 1 year with isoniazid plus PAS, half (selected at random) in a sanatorium and half at home. The incidence of active tuberculosis and of tuberculous infection was no greater in the contacts of patients treated at home than in the contacts of patients treated in a sanatorium, either in the first year or over the subsequent 4 years. The major risk to the contacts resulted from exposure to the patient before diagnosis. These findings reaffirm that close family contacts of patients treated at home were at no additional risk of developing tuberculosis, provided the patients received effective chemotherapy. Finally, this study has shown that it is possible in South India to obtain extremely good cooperation from a group of families over a period of several years.—Authors' Abstract

Manfredi, A. A., Heltai, S., Rovere, P., Sciorati, C., Paolucci, C., Gelati, G., Rugarli, C., Vaiani, R., Clementi, E. and Ferrarini, M. *Mycobacterium tuberculosis* exploits the CD95/CD95 ligand system of gamma delta T cells to cause apoptosis. Eur. J. Immunol. **28** (1998) 1798–1806.

V gamma 9/V delta 2+ T cells specifically recognize *Mycobacterium tuberculosis in vitro* and are precociously recruited in early mycobacterial lesions. Even if gamma delta T cells are only fortuitously detected in granulomas or bronchoalveolar lavages of patients with active pulmonary tuberculosis, a role in shaping the mature alpha beta T-cell response against *M. tuberculosis* is substantiated. Here we provide a molecular explanation for this paradox: the engagement of the gamma delta TCR by mycobacterial antigens induced the expression of CD95 ligand (CD95L) by chronically activated CD95+/CD95L– gamma delta T lymphocytes. The receptor was functional, as CD95/CD95L interaction triggered the bystander death of CD95+ cells by apoptosis. Cell death was abolished by CD95-blocking antibodies. The transient accumulation at the site of infection of CD95L+ gamma delta lymphocytes, capable of interacting with CD95+ leukocytes attracted by the response toward the pathogen, may determine the characteristics of the ensuing granulomatous disease.—Authors' Abstract

Marklund, B.-I., Mahenthiralingam, E. and Stokes, R. W. Site-directed mutagenesis and virulence assessment of the *katG* gene of *Mycobacterium intracellulare.* Mol. Microbiol. **29** (1998) 999–1008.

Mycobacterial catalases have been suggested as acting as virulence factors by protecting intracellular mycobacteria from reactive oxidative metabolites produced by host phagocytes. *Mycobacterium intracellulare,* like many other mycobacteria, produces two proteins with catalase activity: a heat-stable catalase (KatE) and an inducible, heat-labile catalase peroxidase (KatG). The *M. intracellulare katG* gene was cloned, and a plasmid derivative with a 4 bp insertion in the *katG* coding sequence was constructed and used for site-directed mutagenesis of *M. intracellulare* 1403 (ATCC 35761). The resulting *katG* mutant was highly resistant to isoniazid (INH), showed an increased sensitivity to H_2O_2 and had lost peroxidase and heat-sensitive catalase activity but retained heat-stable catalase activity. The plasmid carrying the *katG* frameshift allele was also used for mutagenesis of the mouse virulent *M. intracellulare* isolate D673. After intravenous injection into BALB/c mice, D673 and the isogenic *katG* mutant showed the same growth kinetics in the spleen, liver and lungs of the infected mice. Our results demonstrate that the KatG catalase peroxidase mediates re-

sistance to H_2O_2 and susceptibility to INH but is not an essential virulence factor for the survival and growth of *M. intracellulare* in the mouse.—Authors' Summary

Picardeau, M. and Vincent, V. Mycobacterial linear plasmids have an invertron-like structure related to other linear replicons in *Actinomycetes.* Microbiology **144** (1998) 1981–1988.

The authors previously identified large plasmids in *Mycobacterium xenopi, M. branderi* and *M. celatum* which appeared to have a linear topology. This study has confirmed the presence of such linear plasmids in mycobacteria, including *M. avium,* and demonstrated that the ends of these replicons are covalently bound with protein(s), suggesting an invertron-like structure. The termini of one 25 kb plasmid, designated pCLP, from *M. celatum* were cloned and the first 500 bp of each terminus were sequenced. The termini of this plasmid show the characteristic features of invertrons with terminal inverted repeats of 45 bp (with imperfect matches) and several palindromic sequences. Moreover, similarity existed in the structure and terminal nucleotide sequence of pCLP and the termini of linear replicons of *Streptomyces* and *Rhodococcus* species, indicating a conservation of these linear extrachromosomal elements within the *Actinomycetales.*—Authors' Abstract

Portaels, F., Traore, H., De Ridder, K. and Meyers, W. M. *In vitro* susceptibility of *Mycobacteria ulcerans* in clarithromycin. Antimicrob. Agents Chemother. **42** (1998) 2070–2073.

Buruli ulcer (BU), caused by *Mycobacterium ulcerans,* was recently recognized by the World Health Organization as an important emerging disease. While antimycobacterial therapy is often effective for the earliest nodular or ulcerative lesions, medical management of BU lesions in patients presenting for treatment is usually disappointing, leaving wide surgical excision the only alternative. Advanced ulcerated lesions of BU rarely respond to antimycobacterial agents; however, perioperative administration of such drugs may prevent relapses or disseminated infections. Clarithromycin possesses strong activity *in vitro* and *in vivo* against most nontuberculous mycobacteria. In this study we determined the antimycobacterial activity of this drug *in vitro* against 46 strains of *M. ulcerans* isolated from 11 countries. The MIC of clarithromycin was determined at pH 6.6 (on 7H11 agar) and at pH 7.4 (on Mueller-Hinton agar). The MICs ranged from 0.125 to 2 μg/ml at pH 6.6 and from <0.125 to 0.5 μg/ml at pH 7.4. For the majority of the strains, geographic origin did not play a significant role. Thirty-eight strains (83%) were inhibited by 0.5 μg/ml at pH 7.4. These MICs are below peak therapeutic concentrations of clarithromycin obtainable in blood. These results suggest that clarithromycin is a promising drug both for the treatment of early lesions of *M. ulcerans* and for the prevention of hematogenous dissemination of the etiologic agent during and after surgery. Studies should be initiated to evaluate the effects of clarithromycin in combination with ethambutol and rifampin on *M. ulcerans* both *in vitro* and in experimentally infected mice. Multidrug regimens containing clarithromycin may also help control the secondary bacterial infections sometimes seen in BU patients, most importantly osteomyelitis.—Authors' Abstract

Rastogi, N., Goh, K. S., Horgen, L. and Barrow, W. W. Synergistic activities of antituberculous drugs with cerulenin and trans-cinnamic acid against *Mycobacterium tuberculosis.* FEMS Immunol. Med. Microbiol. **21** (1998) 149–157.

The recent upsurge in the incidence of tuberculosis with significant emergence of multidrug-resistant cases has focused on the priority of discovering effective new drugs and on the strategies to augment the potential of existing drugs against *Mycobacterium tuberculosis.* In the present study, we investigated cerulenin and trans-cinnamic acid, which have recently been shown to augment the activity of various antibiotics against *M. avium* [Antimicrob. Agents Chemother. 38 (1994) 2287–2295], to enhance the activity of isoniazid, ri-

fampin, ofloxacin, amikacin and clofazimine against *M. tuberculosis.* The synergy observed was compared with identical combinations using ethambutol, a cell-wall-inhibiting drug used in standard antituberculous chemotherapy. The results showed that ethambutol resulted in synergistic activity in 12/30 drug combinations, as compared to 15/36 for cerulenin and 10/18 for trans-cinnamic acid. This increase in drug activity was even observed with drug-resistant isolates. Use of novel antimicrobials and understanding of their mechanisms of action may be an effective strategy to determine previously undescribed targets for future drug development.—Authors' Abstract

Rojas, M., Barrera, L. F. and Gardia, L. F. Induction of apoptosis in murine macrophages by *Mycobacterium tuberculosis* is reactive oxygen intermediate-independent. Biochem. Biophys. Res. Commun. **247** (1998) 436–442.

Infection with *Mycobacterium tuberculosis* induces apoptosis in murine macrophage lines. Resistant macrophages B10R (Bcg^r) are more prone to undergo apoptosis than susceptible BIOS (Bcg^s) macrophages. Apoptosis and inhibition of intracellular growth of the mycobacteria seem to be dependent on the production of nitric oxide, since both can be reverted by aminoguanidine (AMG). Although B10R macrophages produce more superoxide anion than BIOS macrophages after infection with *M. tuberculosis,* reactive oxygen intermediate (ROIs) scavengers did not affect uptake of H-3-uracil incorporation by the mycobacteria nor the induction of apoptosis. These results further suggest that both phenomena are dependent on the production of nitric oxide by the infected macrophages.—Authors' Abstract

Segura, C., Salvado, M., Collado, I., Chaves, J. and Coira, A. Contribution of beta-lactamases to beta-lactam susceptibilities of susceptible and multidrug-resistant *Mycobacterium tuberculosis* clinical isolates. Antimicrob. Agents Chemother. **42** (1998) 1524–1526.

The beta-lactamases in 154 clinical *Mycobacterium tuberculosis* strains were studied. Susceptibilities to beta-lactam antibiotics, their combination with clavulanate (2:1), and two fluoroquinolones were determined in 24 *M. tuberculosis* strains susceptible to antimycobacterial drugs and in nine multiresistant strains. All 154 *M. tuberculosis* isolates showed a single chromosomal beta-lactamase pattern (pI 4.9 and 5.1). *M. tuberculosis* beta-lactamase hydrolyzes cefotaxime with a maximum rate of 22.5 ± 2.19 IU/liter (strain 1382). Neither amoxicillin, carbenicillin, cefotaxime, ceftriaxone, nor aztreonam was active alone. Except for aztreonam, beta-lactam combinations with clavulanate produced better antimycobacterial activity.—Authors' Abstract

Senaratne, R. H., Mobasheri, H., Papavinasasundaram, K. G., Jenner, P., Lea, E. A. and Draper, P. Expression of a gene for a porin-like protein OmpA family from *Mycobacterium tuberculosis* H37Rv. J. Bacteriol. **180** (1998) 3541–3547.

An open reading frame in the genomic database of *Mycobacterium tuberculosis* H37Rv was identified as having homolog with an outer membrane protein. We found that the gene specified a protein belonging to the OmpA family, which includes some porins of gram-negative organisms. The gene was amplified by PCR and cloned into *Escherichia coli.* Overexpression of the gene was toxic to the host, but limited amounts could be purified from cells before growth ceased. A truncated gene devoid of the code for a presumed signal sequence was well expressed, but the protein had no pore-forming activity in the liposome swelling assay. However, the intact protein, OmpATb, behaved as a porin of low specific activity, with a pore diameter of 1.4 to 1.8 nm, and was also active in planar lipid bilayers, showing a single-channel conductance of 700 pS. The protein had a molecular mass of about 38 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A polyclonal rabbit antiserum raised to the truncated protein recognized a protein of similar molecular mass in detergent extracts of broken *M. tuberculosis* cells. Reverse

transcription-PCR confirmed that the gene for OmpATb was expressed in *M. tuberculosis* cells growing in culture. Comparison of the purified protein with that in the detergent-extracted preparation using liposomes and planar lipid bilayers showed that the two materials had similar pore-forming properties. OmpATb is different from either of the mycobacterial porins described so far. This is the first report of a porin-like molecule from *M. tuberculosis*; the porin is likely to be important in controlling the access of hydrophilic molecules to the bacterial cell.—Authors' Abstract

Tsenova, L., Sokol, K., Freedman, V. H. and Kaplan, G. A combination of thalidomide plus antibiotics protects rabbits from mycobacterial meningitis-associated death. J. Infect. Dis. **177** (1998) 1563–1572.

Tuberculous meningitis (TBM) is a devastating form of tuberculosis that occurs predominantly in children and in immunocompromised adults. To study the pathogenesis of TBM, a rabbit model of acute mycobacterial central nervous system infection was set up (8-day study). Inoculation of live *Mycobacterium bovis* Ravenel intracisternally induced leukocytosis (predominantly mononuclear cells), high protein levels, and release of tumor necrosis factor-alpha (TNF-α) into the cerebrospinal fluid within 1 day. Histologically, severe meningitis with thickening of the leptomeninges, prominent vasculitis, and encephalitis was apparent, and mortality was 75% by day 8. In animals treated with antituberculous antibiotics only, the inflammation and lesions of the brain persisted despite a decrease in mycobacteria; 50% of the rabbits died. When thalidomide treatment was combined with antibiotics, there was a marked reduction in TNF-α levels, leukocytosis, and brain pathology. With this combination treatment, 100% of the infected rabbits survived, suggesting a potential clinical use for thalidomide in TBM.—Authors' Abstract

U.S.A. Advisory Council for the Elimination of Tuberculosis (ACET). Development of new vaccines for tuberculosis; recommendations of the ACET. MMWR **47** (1998) 1–6.

Tuberculosis (TB) remains a major, global public health problem, particularly in low-income countries. Better application of current diagnostic, treatment, and prevention strategies could lead to gradual decreases in the disease, but eliminating TB completely in the United States and internationally will require new tools. The greatest impact could come from a new vaccine, and recent technological advances have provided the basis for new vaccine development. However, sustained support is required to move the research from the laboratory to field trials of vaccines and to implement new vaccine programs. Recognizing the importance of TB vaccines, the Advisory Council for the Elimination of Tuberculosis (ACET) recommends that public agencies and vaccine manufacturers develop a comprehensive, consensual strategy to achieve these goals. This report outlines the elements that should be considered in devising a strategic plan for vaccine development.—Authors' Summary

Voladri, R. K. R., Lakey, D. L., Hennigan, S. H., Menzies, B. E., Edwards, K. M. and Kernodle, D. S. Recombinant expression and characterization of the major beta-lactamase of *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **42** (1998) 1375–1381.

New antibiotic regimens are needed for the treatment of multidrug-resistant tuberculosis. *Mycobacterium tuberculosis* has a thick peptidoglycan layer, and the penicillin-binding proteins involved in its biosynthesis are inhibited by clinically relevant concentrations of beta-lactam antibiotics. Beta-lactamase production appears to be the major mechanism by which *M. tuberculosis* expresses beta-lactam resistance. Beta-lactamases from the broth supernatant of 3- to 4-week-old cultures of *M. tuberculosis* H37Ra were partially purified by sequential gel filtration chromatography and chromatofocusing. Three peaks of beta-lactamase activity with pi values of 5.1, 4.9, and 4.5, respectively, and which accounted

for 10%, 78%, and 12% of the total post-chromatofocusing beta-lactamase activity, respectively, were identified. The beta-lactamases with pi values of 5.1 and 4.9 were kinetically indistinguishable and exhibited predominant penicillinase activity. In contrast, the beta-lactamase with a pi value of 4.5 showed relatively greater cephalosporinase activity. An open reading frame in cosmid Y49 of the DNA library of *M. tuberculosis* H37Rv with homolog to known class A beta-lactamases was amplified from chromosomal DNA of *M. tuberculosis* H37Ra by PCR and was overexpressed in *Escherichia coli*. The recombinant enzyme was kinetically similar to the pi 5.1 and 4.9 enzymes purified directly from *M. tuberculosis*. It exhibited predominant penicillinase activity and was especially active against azlocillin. It was inhibited by clavulanic acid and m-aminophenylboronic acid but not by EDTA. We conclude that the major beta-lactamase of *M. tuberculosis* is a class A beta-lactamase with predominant penicillinase activity. A second, minor beta-lactamase with relatively greater cephalosporinase activity is also present.—Authors' Abstract