# CURRENT LITERATURE

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

## General and Historical

**Parra, M. C.** [A socioeconomic characterization of leprosy patients at the dermatology clinic in Maracaibo, Venezuela; a case study.] Cad. Saude Publica **12** (1996) 225–231. (in Spanish)

This paper presents the socioeconomic characteristics of leprosy patients treated at the Dermatology Clinic in Maracaibo, Venezuela (UDS). The characteristics were obtained from a closed questionnaire given to 40 patients. Results indicate that this is mainly an adult male population, with a reasonable level of schooling who both work and belong to apparently well-established, stable family groups; their family income levels correspond to lower or medium-low social class groups. In addition, the patients are mainly nondisabled, and clinical diagnoses are mostly of the lepromatous and borderline types. Any educational program targeting this group should take these socioeconomic characteristics into account in defining the kind of patients who receive treatment at UDS.—Trop. Dis. Bull. **94** (1997) 39

## Chemotherapy

**Bakirtzief, Z.** [Obstacles to compliance with treatment for Hansen's disease.] Cad. Saude Publ. **12** (1996) 497–505. (in Spanish)

This research project aimed at identifying some of the factors related to leprosy patient compliance with the multidrug treatment regimen. The methodological framework of the Social Representations Theory was used. Two groups of patients were interviewed: compliant and noncompliant with treatment and those coming from two different health services. We observed a common understanding about treatment in the various interviews, expressed as a metaphor to describe the treatment experience: the figure of a battle in which the bacillus is portrayed as a threat, the patient as a victim, the medication as a weapon and the health professional as a hero or saint. Still, the medication is represented as being both good and bad for the patient's well-being. Finally, the quality of the physician-patient relationship appeared to be the main difference between the two groups of subjects studied.—Author's English Abstract

**de Oliveira, M. L. W.** [Cure of hanseniasis and the magnitude of relapses.] An. Bras. Dermatol. **72** (1997) 63–69. (in Portuguese)

The cure of the multibacillary form of leprosy after 24 doses of multidrug therapy is still a controversial subject. All retrospective reports with different therapeutic schemes show successful results. Further analysis indicates that relapse rate is correlated with a high bacterial load and delayed diagnosis. Monotherapy with any of the available drugs favors relapse. Relapse rates from current reports reach 0.23 to 3.3/100 patients/year of observation. It is known that multidrug therapy efficacy is little affected with less than 10% relapse rates, due to bacillary resistance or persistence, and all efforts must be made in order to increase treatment regularity as well as early diagnosis.—Author's English Summary

**Mahmud, R., Tingle, M. D., Maggs, J. L., Cronin, M. T. D., Dearden, J. C. and Park, B. K.** Structural basis for the haemotoxicity of dapsone: the importance of the sulphonyl group. Toxicology **117** (1997) 1–11.

The structural basis of dapsone (4,4′-diaminodiphenyl sulfone) hemotoxicity has been determined by investigation of the *in vitro* bioactivation of a series of 4-substituted arylamines. In the presence of rat river microsomes, dapsone (100 $\mu$M) was the most potent former of methemoglobin in human erythrocytes (44.8 ± 6.7%). Substitution of the sulfone group with sulfur (11.6 ± 1.4% methemoglobin), oxygen (4.5 ± 1.1%), nitrogen (0.0 ± 3.2%), carbon (13.6 ± 0.8%) or a keto group (34.0 ± 6.1%) resulted in a decrease in methemoglobin formation. Only one compound, 4,4′-diaminodiphenylamine, generated significant ($p < 0.001$) amounts of methemoglobin (25.6 ± 2.5%) in the absence of NADPH. To assess further the role of the 4-substituent in methemoglobinemia, the toxicity of a series of 4-substituted aniline derivatives was also studied. Of the anilines studied, 4-nitroaniline caused the most methemoglobin (36.5 ± 8.0%), while aniline caused the least (0.3 + 0.5%). Overall, there was a significant correlation ($r^2 = 0.83$) between the hemotoxicity and the Hammett constant, sigma (p), suggesting that it is the electron-withdrawing properties of the substituent that influence the methemoglobin formation. In the presence of microsomes prepared from two human livers, dapsone was the most hemotoxic bis arylamine, whereas 4-iodoaniline was the most potent methemoglobin former (60.6 and 73.6%) and aniline the least potent (1.1 and 2.4%). As a whole, these results indicate that the sulfonyl group, which is essential for the pharmacological activity of dapsone, is also largely responsible for the hemotoxicity seen with this drug.—Authors' Abstract

**Rao Mamidi, N. V. S., Prabhakar, M. C. and Krishna, D. R.** Disposition of rifampicin following intranasal and oral administration. Indian J. Lepr. **68** (1996) 149–153.

*Mycobacterium leprae* are lodged in the noses of patients with multibacillary leprosy. Rifampin, a potent bactericidal antileprotic drug, is given orally to such patients with a view to making the infective cases noninfective. Earlier work by the authors has shown that intranasal administration of rifampin helps to reduce the *M. leprae* load in the nose much faster than after conventional oral administration. In the present study from India, rifampin concentrations in plasma/urine/nasal wash of healthy volunteers following oral and intranasal administration were determined. Following intranasal administration, rifampin was not detectable in plasma and high concentrations were measured in the nasal wash. Following oral administration, rifampin was not detectable in the nasal wash, indicating that sufficient concentrations of this drug are not available for clearing *M. leprae* from the nose.—Trop. Dis. Bull. **93** (1996) 1040

**Subcommittee on Clinical Trials of the Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.** Response to treatment by multidrug regimens in the THELEP controlled clinical drug trials. Lepr. Rev. **67** (1996) 260–279.

During the period 1977–1983, clinical trials of five multidrug regimens were conducted among 215 patients with previously untreated multi-bacillary leprosy at the Institut Marchoux, Bamako, Mali, and the Central Leprosy Teaching and Research Institute, Chingleput, South India. The trials were designed primarily to permit measurement of the proportions of persisting *Mycobacterium leprae* in the patients' skin lesions. In addition, the combination of the large number of patients studied, the large volume of carefully standardized data, and the employment of multidrug regimens provided a unique opportunity to measure the clinical response of patients to treatment by these regimens.

Persisting *M. leprae* were detected in 7.8% of all specimens; the frequency did not vary with center, regimen, or duration of treatment. The bacterial index (BI) decreased by a mean annual rate of 75%, the logarithmic biopsy index by a mean annual rate of

87%, and the logarithm$_{10}$ number of acid-fast bacilli per g tissue by a mean annual rate of 69%. The rate of decrease of these measures of the numbers of *M. leprae* was related to the "strength" of the regimen.

Although no difference of clinical status as a function of regimen was demonstrated, a difference was observed between the two centers, probably the result of different clinical criteria employed by the responsible physicians. A change of histopathological classification in the course of the trials was recorded for 12% of the patients, most representing upgrading from LL to BL, without relation to regimen or treatment center.

Erythema nodosum leprosum (ENL) was less severe for the patients treated by the maximal regimen in Chingleput, which included daily clofazimine; as expected, the majority of patients treated by this regimen were found to have maximal pigmentation. Prednisolone was evidently preferred for treatment of ENL in Chingleput; whereas thalidomide was preferred in Bamako.

Fourteen cases of jaundice were observed, primarily among the patients treated by the maximal regimens, that included daily administration of rifampin for the entire 2 years of the trials. Measurements of weight and blood pressure, and studies of the blood and of hepatic and urinary tract functions revealed only negligible differences among regimens and between centers. In many cases, those differences that were observed were associated with ENL.—Authors' Abstract

**Vijayakumaran, P., Manimozhi, N., Jesudasan, K., Arunthathi, S., Jacob, M. and Samuel, P.** Leucocytopenia after rifampicin and ofloxacin therapy in leprosy. Lepr. Rev. **68** (1997) 10–15.

New antimycobacterial agents and combined treatment regimens are being introduced for the treatment of leprosy. Ofloxacin is one such broad spectrum antimicrobial agent. In this study rifampin plus ofloxacin were administered daily for 4 weeks (daily supervised dose). Two patients (and possibly a third patient who refused all investigations) out of 125 patients developed leukocytopenia during the third week of therapy. It was associated with fever, malaise, nausea and loss of appetite. They recovered after cessation of drug treatment. Patients receiving ofloxacin should be monitored for constitutional symptoms suggestive of this complication even though the risk of such complication may be minimal.—Authors' Summary

**Wang, H.-Y., Shi, M.-Q. and Wang, H.-C.** [Activity of combinations of effective drugs against *Mycobacterium leprae* in nude mice.] Chin. J. Dermatol. **29** (1996) 25–26.

With the objective of improving multidrug treatment (MDT) regimens for leprosy patients, the activity of combinations of several new bactericidal drugs against *Mycobacterium leprae* was studied in nude mice. *M. leprae*-infected nude mice were treated from day 84 to 179 after inoculation. Three regimens were evaluated: minocycline (M) 0.01% + dapsone (D) 0.001%; M + D + ofloxacin (O) 0.05%; M + D + rifampin (R) 0.01% in the diet. The results indicated that there was additive or synergistic activity against *M. leprae*; furthermore, a combination of 3 effective drugs was confirmed to be superior to that of 2 drugs. The bactericidal activity of M + D + R was the best of these regimens: 5 months after stopping therapy, no multiplication of *M. leprae* was observed in the hind foot pads of inoculated nude mice.—Trop. Dis. Bull. **93** (1996) 1041

## Clinical Sciences

**Agdamag, A. T., Endoh, M., Kawatu, K. and Izumi, S.** A serologic *Mycobacterium leprae* gelatin particle agglutination (MLPA) in the diagnosis of leprosy: comparison with conventional enzyme-linked immunoassay and bacterial index. Jpn. J. Lepr. **65** (1996) 100–105.

A gelatin particle agglutination test (MLPA) for the detection of antiphenolic

glycolipid-I (PGL-I) antibodies was compared with the slit-skin smear method in the diagnosis of leprosy in the Philippines. MLPA and BI tests showed a good agreement rate of 88.1% and MLPA and ELISA tests showed an excellent agreement rated of 96.2%. This MLPA test is concluded to be simple and reliable, and so will be very convenient for medical practitioners and of great benefit for leprosy patients.—Trop. Dis. Bull. **94** (1997) 129

**Castellazzi, Z., Bogaert, H., Isa, R. and Ledesma, R.** [Incidence of reactional episodes in a group of leprosy patients treated with polychemotherapy.] Rev. Dominicana Dermatol. **23** (1996) 15–21. (in Spanish)

A study was made on Jopling's type 1 and 2 reactional episodes that occurred on 801 leprosy patients belonging to the LL, BB and BT forms diagnosed at the Dermatologic Unit of the National District during the period 1984–1995 and treated with polychemotherapy. Different variations were analyzed to study the behavior of those epiphenomenums, and it was observed that in addition to such reduction from type 2 reactions, a small increase occurred in reactional episodes type 1. The results of our investigation were compared with those reported in a study performed until the year 1983 done by Bogaert and Pittaluga on leprosy patients of LL at the IDCP.—Authors' English Summary

**Chen, W., *et al.*** [On the causes of blindness and lower vision in leprosy.] China Lepr. J. **12** (1996) 251–253. (in Chinese)

During the period of 1985 to 1990, the vision of 2229 inpatients in leprosaria was examined. The results showed that the blindness rate was 15.4% and lower vision rate was 16.2% in them, and their causes are mainly lagophthalmos, ectropion, iridocyclitis and cataract. To prevent blindness in leprosy patients we must detect and treat these eye conditions in time.—Authors' English Abstract

**Chen, Y.** [Treatment of glaucoma caused by iridocyclitis in leprosy.] China Lepr. J. **12** (1996) 250–251. (in Chinese)

Secondary glaucoma caused by iridocyclitis was found in seven eyes of five leprosy patients with a mean age of 63.6, including 3 BB and 2 TT. Six eyes in the five cases have healed after treatment with drugs lowering intraocular pressure, cortisone and topical application of atropine solution, so their vision increased from seeing finger movement or counting fingers to 0.08 ~ 0.5. The author pointed out that it is essential to actively treat iridocyclitis for the prevention of glaucoma.—Author's English Abstract

**Gimenez, R. M. F., Grassi, M. L. and Molinari, G. M. L.** [Reactional episodes in leprosy.] Dermatol. Rev. Mex. **40** (1996) 101–105. (in Spanish)

The authors describe the various leprosy reactions that may develop during the chronic course of the disease or in untreated patients, and then present their findings in 90 patients with leprosy reactions seen at the Dermatological Centre "Dr. M. M. Giménez" in Resistencia, Argentina. They discuss their experience with Langerhans' cells in some patients. It is stated that the secret of successful management of leprosy reactions is early diagnosis and the timely initiation of treatment.—Trop. Dis. Bull. **93** (1996) 1035

**Girma, T., Mengistu, F. and Hogeweg, M.** The pattern of cataract and the postoperative outcome of cataract extraction in Ethiopian leprosy patients as compared to nonleprosy patients. Lepr. Rev. **67** (1996) 318–324.

Cataract is a blinding disease occurring all over the world. One of the causes of cataract is leprosy.

Sixty leprosy and 100 nonleprosy patients were assessed and underwent intracapsular cataract extraction.

Leprosy patients with cataract were much younger than nonleprosy patients. The leprosy group had a significantly higher rate of complications, and this was seen more in paucibacillary cases. There was a higher rate of visual disability in the leprosy group than in the nonleprosy group.

Cataract was seen in younger patients in the leprosy group. This raised the possibil-

ity of leprosy being the cause of the cataract. The leprosy group consisted mostly of multibacillary cases; however, unlike in other studies, the rate of complications tended to be higher in the paucibacillary group. There were no pre-operative findings that correlated with a low postoperative intraocular pressure.—Authors' Summary

**Guerrero, P., Castellazzi, Z., Bogaert, J. and Isa, R.** [Ocular leprosy: a study of 238 patients under ambulatory control, Dominican Republic.] Rev. Dominicana Dermatol. **22** (1995) 13–19. (in Spanish)

An investigation was made on the ocular pathology caused by leprosy in 238 patients controlled in an ambulatory manner in the IDCP; 64.7% of these patients presented some type of ocular injury. These appeared more frequently as the time lapse became more extensive for the diagnosis of the ailment. The most frequent signs in the anterior pole of the ocular globe were the enlargement of the nerves in cornea, corneal anesthesia and pannus. In the structures annexed to the ocular globe were madarosis, trichiasis, hypotrichosis, and lagophthalmos.—Authors' English Summary

**Kumar, G. R., Ramana, P. V., Vasundhara, N. and Reddy, M. K.** Two unusual nerve abscesses—lepromatous leprosy and pure neural leprosy: case reports. Lepr. Rev. **67** (1996) 217–221.

The authors report on two cases of nerve abscesses, one associated with lepromatous leprosy (LL) and the other with tuberculoid neural leprosy, seen in Andhra Pradesh, India. Neither had any signs of reactions. Both were untreated cases. Surgical nerve decompression and systemic prednisolone resolved the nerve abscess in the first case, whereas the second one responded only to surgical nerve decompression. The unusual nature of the clinical presentations of nerve abscess was outlined.—Trop. Dis. Bull. **94** (1994) 41

**Mahajan, P. M., Jogaikar, D. G. and Mehta, J. M.** A study of pure neuritic leprosy: clinical experience. Indian J. Lepr. **68** (1996) 137–141.

Manifestations of leprosy in pure neuritic form accounted for 179 patients out of the total 3853 leprosy patients (4.6%) attending the Poona Urban Leprosy Investigation Centre clinics in India. Patients with pure neuritic leprosy are prone to develop nerve damage. Eighty-seven (48.6%) of the pure neuritic patients presented with deformities. Involvement of the upper extremity and right ulnar nerve in particular was the most common clinical feature. Patients presenting with involvement of two nerves of the same extremity were also quite common. None of the patients developed skin lesions while on antileprosy treatment. The authors conclude that it is important to recognize neuritic symptoms early and suspect leprosy even in the absence of skin lesions.—Trop. Dis. Bull. **93** (1996) 1037

**Qian, G.** [Canceration of chronic ulcers in the leg in leprosy.] China Lepr. J. **12** (1996) 246–248. (in Chinese)

Since June 1980 to November 1993, ten cases of lower limb ulcers with canceration were found, including six in the sole, three in the leg and one in the ankle. They are eight men and two women with a mean age of 57 and mean ulceration duration of 17 years. All of them had been amputated at the level of the thigh. Three and two cases died 1 year and 4 to 5 years after the operation, respectively, because of squamous epithelial carcinoma metastasis or other cancers; two are living and three left without contact.—Authors' English Abstract

**Ramesh, V. and Porichha, D.** Practical problems in the management of leprosy. Lepr. Rev. **67** (1996) 330–336.

The categorization of leprosy into paucibacillary or multibacillary depends on the report of slit-skin smears. Unfortunately, in many control programs the quality of slit smears is below par. Taking the example of India, the main reasons were that the work of laboratory technicians was unrewarding as compared to serving in a general health care system. There was lack of equipment

and an unrealistic patient-to-technician ratio. Future attempts were made by experienced workers to devise a clinical system for classifying leprosy as paucibacillary (PB) or multibacillary (MB) based on counting the number of lesions. However this method did not prove cost-effective because more PB patients were classified in the MB group, increasing the burden of treatment. A renewed attempt to improve slit-smear performance should be made by modifying the existing methods. This can definitely improve the situation. Patients with multiple macular lesions and those with neuritic leprosy are best treated with the MB-MDT regimen. The treatment for PB leprosy is to continue up to 6 months but in MB leprosy with a high bacterial index a longer duration of MDT may be required. Following completion of MDT many cases with deformity are accumulating and their care forms a neglected part of many control programs. In addition to strengthening the infrastructure, simple techniques must be imparted to those with deformities and disabilities. This involves the artful and innovative cooperation of the health worker, patient and the community. The leprosy worker should be motivated to promote such activities.—Authors' Summary

**Richardus, J. H., Finlay, K. M., Cròft, R. P. and Smith, W. C. S.** Nerve function impairment in leprosy at diagnosis and at completion of MDT: a retrospective cohort study of 786 patients in Bangladesh. Lepr. Rev. **67** (1996) 297–305.

This retrospective cohort study includes all new leprosy patients registered for multidrug therapy (MDT) in 1990 at the Danish-Bangladesh Leprosy Mission project in Bangladesh. The main objective was to determine the extent of nerve function impairment (NFI) at diagnosis and at completion of MDT, and to identify opportunities for intervention and their relative impact on the prevention of disabilities (POD).

A total of 786 patients were included; 486 males and 300 females. There were 315 paucibacillary (PB) and 471 multibacillary (MB) patients. In terms of the WHO leprosy disability grading system, at the time of diagnosis 31/315 (9.8%) had grade 1 or grade 2 disability in the PB group, and 177/471 (37.6%) in the MB group. The incidence rate of NFI during MDT was 3.5 per 100 person years at risk (PYR) in the PB group, and 7.5 per 100 PYR in the MB group. In the MB group 37 (7.9%) previously normal patients sustained NFI during MDT, while 19 (4.0%) with NFI at diagnosis showed complete recovery at completion of MDT. The most commonly involved nerves were the ulnar (motor function) and the posterior tibial nerves (sensibility). Reversal reactions were observed in 0.6% of the PB patients during MDT, giving an incidence rate of 1 per 100 PYR. The percentage of MB patients diagnosed with reversal during MDT was 14.2%, giving an incidence rate of 6 per 100 PYR. The percentage of MB patients diagnosed with ENL during MDT was 2.1%, with an incidence rate of 1 per 100 PYR.

It was concluded that early detection of new cases of leprosy would prevent disabilities in more than 30% of all patients, thus having the highest impact in the quest for the prevention of disabilities (POD). POD activities during and after MDT will prevent disabilities in approximately 10% of all cases. This study also indicates that treatment with prednisolone is effective and should be available at field level for all patients with recent NFI.—Authors' Abstract

**Shen, J., *et al.*** [Lepra reaction and its related factors.] China Lepr. J. **12** (1996) 234–237. (in Chinese)

Possible influences of ages, sex, bacterial index (BI) and MDT on type 1 and 2 lepra reactions were studied in 478 newly diagnosed cases of leprosy from 1983 to 1993. Of 428 BT-BL cases 41 (8.6%) had type 1 reaction and of 218 BB-LL cases 29 (13.3%) developed type 2 reaction. The high risk factors are BI of 0.1 to 0.3, the period of 1 to 6 months after MDT and BT-BB forms of leprosy for type 1, and leprosy onset in adolescence, BI of 3.0–5.0, the period of 4 to 6 months after MDT and BL-LL forms of leprosy for type 2 reaction. The authors think that to know these will be conducive to the prevention and control of lepra reaction.—Authors' English Abstract

**Soares, D. and Kimula, Y.** Squamous cell carcinoma of the foot arising in chronic ulcers in leprosy patients. Lepr. Rev. **67** (1996) 325–329.

Squamous cell carcinoma (SCC) of the foot is a rare sequelae of chronic ulceration secondary to leprosy neuropathy. Most of the tumors are relatively slow growing and tend to metastasize late. Survival after local excision is generally good. In this series of 17 patients so far there have been 3 deaths attributable to SCC, all of whom presented with locally advanced tumors and lymph node metastasis.—Authors' Summary

**Soares, D. and Riedel, A.** A simple and inexpensive pinch meter to detect subclinical weakness among leprosy patients. Lepr. Rev. **68** (1997) 55–60.

This paper describes the use of a neonatal sphygmomanometer cuff as a simple, inexpensive pinch meter. Normal values for key pinch, pulp pinch and side pinch in the dominant hand of healthy Nepali people are provided. The pinch meter was also used to test pinch strength in hands affected by leprosy and normal hands. Some patients with leprosy who have no objective weakness on voluntary muscle testing (VMT) have less pinch strength than people without leprosy. The pinch meter is a useful tool for the early detection of motor function loss.—Authors' Summary

**Thappa, D. M., Garg, B. R., Rao, M. V. and Gharami, R.** Impact of HIV infection on leprosy. Indian J. Lepr. **68** (1996) 255–256.

The clinical process of leprosy in an HIV-infected patient with associated latent syphilis and condyloma acuminata in Bombay, India, is described. Even with adequate multidrug therapy, the patient continued to develop new leprosy patches, and neurological deterioration occurred in spite of the addition of pefloxacin.—Trop. Dis. Bull. **94** (1997) 129

**van Brakel, W. H., Kets, C. M., van Leerdam, M. E., Khawas, I. B. and Gurung, K. S.** Functional sensibility of the hand in leprosy patients. Lepr. Rev. **68** (1997) 25–37.

The aims of this cross-sectional comparative study was to compare the results of Semmes–Weinstein monofilament (SWM) testing and moving 2-point discrimination (M2PD) with four tests of functional sensibility: recognition of objects, discrimination of size and texture and detection of dots.

Ninety-eight leprosy in- and outpatients at Green Pastures Hospital in Pokhara, Nepal, were tested with each of the above tests and the results were compared to see how well they agreed. Using the tests of functional sensibility as reference points, we examined the validity of the SWM and M2PD as predictors of functional sensibility.

There was definite, but only moderate correlation between thresholds of monofilaments and M2PD and functional sensibility of the hand. A normal result with the SWM and/or M2PD had a good predictive value for normal functional sensibility. Sensitivity was reasonable against recognition of objects and discrimination of textures as reference tests (80%–90% and 88%–93%), but poor against discrimination of size and detection of dots (50%–75% and 43%–65%). Specificity was high for most combinations of SWM or M2PD with any of the tests of functional sensibility (85%–99%). Above a monofilament threshold of 2 g, the predictive value of an abnormal test was 100% for dot detection and 83%–92% for textural discrimination. This indicates that impairment of touch sensibility at this level correlates well with loss of dot detection and textural discrimination in patients with leprous neuropathy. For M2PD the pattern was very similar. Above a threshold of 5 mm, 95%–100% of affected hands had loss of dot detection and 73%–80% had loss of textural discrimination.

Monofilament testing and M2PD did not seem suitable as proxy measures of functional sensibility of the hand in leprosy patients. However, a normal threshold with monofilaments and/or M2PD had a good predictive value for normal functional sensibility. Above a monofilament threshold of 2 g and/or a M2PD threshold of 5 mm, textural discrimination was abnormal in most hands.—Authors' Summary

**Wilder-Smith, E., Wilder-Smith, A., van Brakel, W. H. and Egger, M.** Vasomotor reflex testing in leprosy patients, healthy contacts and controls: a cross-sectional study in Western Nepal. Lepr. Rev. **67** (1996) 306–317.

Objective: To examine test characteristics of laser Doppler vasomotor reflex testing for leprosy and to determine the prevalence of abnormal responses in leprosy patients, healthy contacts and controls.

Design and participants: Cross-sectional study including 89 leprosy patients (mean age 35 years, 74% male), 36 healthy contacts (29 years, 64% male) and 47 controls (30 years, 68% male), for a total of 172 participants.

Setting: Leprosy hospital in an endemic region 200 km west of Kathmandu, Nepal.

Outcome measure: Finger-tip and toe-tip vasomotor reflexes elicited by inspiratory gasp were measured using a laser-Doppler flow temperature technique. Results were expressed in per cent as the maximal reduction in bloodflow from baseline.

Results: For all 12 measurement sites there were highly significant ($p < 0.0001$ to $< 0.004$) differences between the three groups tested. Leprosy patients consistently had the lowest responses and controls the highest, with healthy contacts showing intermediate values. Thresholds defined as mean bloodflow reductions among controls minus 1.64 or minus 1.96 standard deviations provided optimal combinations of sensitivity and specificity. Using these cut-off values around 80% of leprosy patients, 50% of healthy contacts and 20% of controls had two or more abnormal reflexes ($p < 0.0001$ for differences between groups).

Conclusions: In endemic regions, subclinical autonomic neuropathy may be an early but detectable marker for the risk of subsequent leprosy, making early treatment and prevention of transmission possible. Prospective studies are needed to establish the predictive value of abnormal vasomotor reflexes.—Authors' Summary

## Immuno-Pathology

**Adams, E., Basten, A., Rodda, S. and Britton, W. J.** Human T-cell clones to the 70-kilodalton heat shock protein of *Mycobacterium leprae* define mycobacterium-specific epitopes rather than shared epitopes. Infect. Immun. **65** (1997) 1061–1070.

The mycobacterial 70-kDa heat shock protein (hsp70) is a dominant antigen during the human T-cell response to mycobacterial infection despite the conserved sequence with the human homolog. To determine whether this response is pathogen specific, CD4+ T-cell clones were isolated from *Mycobacterium leprae* hsp70-reactive individuals. The cytokine profile of the clones was mixed, with all of the clones releasing interferon gamma and half releasing interleukin-4 on stimulation, while six demonstrated cytolytic activity. Five clones reacted with the N-terminal half of the molecule, and the epitopes identified were mycobacterium specific. Residues 241 to 260 were identified by three clones, one of which was restricted by HLA-DR7 (DR7), while a DR1-restricted clone identified residues 71 to 90 and residues 261 to 280 were recognized in the context of DR3. The remaining five T-cell clones reacted with the C-terminal half of the molecule, and the precise position of these epitopes was mapped with 12-mer peptides overlapping by 11 residues. Two of these clones identified overlapping epitopes from residues 411 to 425 and 412 to 428, the latter restricted by DR3. Further epitopes were mapped to residues 298 to 313 restricted by DRw53, residues 388 to 406 restricted by DRw52 or DQ2, and residues 471 to 486 restricted by DR1. The sequences of three epitopes, residues 411 Eo 425, 412 to 428, and 471 to 486, showed significant identity with the equivalent regions of the prototype human hsp70. However, when amino acid substitutions that made the sequence more like the human sequence were introduced, the changes were tolerated poorly as measured by proliferation, cytokine production, and cytotoxic potential. Therefore, T-cell recog-

nition of the *M. leprae* hsp70 antigen occurs in the context of multiple HLA-DR phenotypes and is exquisitely species specific.—Authors' Abstract

**Adams, L. B., Gillis, T. P., Hwang, D. H. and Krahenbuhl, J. L.** Effects of essential fatty acid deficiency on prostaglandin $E^2$ production and cell-mediated immunity in a mouse model of leprosy. Infect. Immun. **65** (1997) 1152–1157.

Results from animal and *in vitro* studies suggest that essential fatty acid (EFA) deficiency enhances cell-mediated immunity by reducing production of prostaglandins with immunosuppressive actions. However, direct experimental evidence that EFA deficiency enhances T-lymphocyte function *in vivo* has not been obtained. In this study, athymic (nu/nu) mice were infected in the foot pads with *Mycobacterium leprae* and fed a linoleic acid-free diet. These mice, and infected nu/nu mice on control diets, were given an adoptive transfer of *M. leprae*-primed, T-cell-enriched lymphocytes. After 2 weeks, *M. leprae* bacilli were harvested from the recipient mice and bacterial viability was determined by the BACTEC system. *M. leprae* recovered from recipient mice fed control diets displayed little reduction in metabolic activity. In contrast, *M. leprae* from recipient mice fed the EFA deficient (EFAD) diet exhibited markedly reduced viability. *In vitro,* donor cells from *M. leprae*-primed mice secreted elevated levels of gamma interferon upon exposure to the bacilli. These cells also exhibited an enhanced proliferative response, which was reduced by exogenous prostaglandin $E^2$ ($PGE^2$). In addition, *M. leprae*-infected granuloma macrophages from EFAD recipient nu/nu mice secreted significantly less $PGE^2$ than granuloma macrophages from mice on control diets. These data suggest that enhanced levels of macrophage-generated $PGE^2$, induced by *M. leprae* or its constituents, could act as an endogenous negative modulator of the immune response occurring in the microenvironment of the lepromatous granuloma.—Authors' Abstract

**Barker, L. P., George, K. M., Falkow, S. and Small, P. L. C.** Differential trafficking of live and dead *Mycobacterium marinum* organisms in macrophages. Infect. Immun. **65** (1997) 1497–1504.

We characterized the *Mycobacterium marinum* phagosome by using a variety of endocytic markers to follow the path of the bacteria through a mouse macrophage cell line. Using a laser nonfocal microscope, we found that the majority of viable *M. marinum* cells were in nonacidic vacuoles that did not colocalize with the vacuolar proton ATPase (V-ATPase), the calcium-independent mannose-6-phosphate receptor (CI-M6PR), or cathepsin D. In contrast, heat-killed organisms and latex beads were in acidic vacuoles which contained the V-ATPase, the CI-M6PR, and cathepsin D. A population of vesicles that contained live *M. marinum* labeled with the lysosomal glycoprotein LAMP-1, but the percentage of vacuoles that labeled was lower than for heat-killed organisms or latex beads. When testing live and heat-killed *M. tuberculosis,* we found levels of colocalization with LAMP-1 and cathepsin D comparable to those for the *M. marinum* isolate. We conclude that *M. marinum,* like *M. tuberculosis,* can circumvent the host endocytic pathway and reside in an intracellular compartment which is not acidic and does not fuse with lysosomes. In addition, we describe a system for sampling a large population of intracellular organisms using a laser confocal microscope.—Authors' Abstract

**Blackwell, J. M.** Structure and function of the natural-resistance-associated macrophage protein (Nramp1), a candidate protein for infectious and autoimmune disease susceptibility. Mol. Med. Today **2** (1996) 205–211.

*Nramp1,* a gene originally identified as *Ity/Lsh/Bcg* for its role in controlling *Salmonella typhimurium, Leishmania donovani* and *Mycobacterium bovis* infections in mice, regulates a cascade of gene-inductive events mediating inflammation, elimination of the invading organism and induction of T-cell memory against re-invasion. How the structure of the Nramp1 protein might relate to its function, and how variable expression of the human homolog (*NRAMP1*) might mediate enhanced resistance to infec-

tion, but cause susceptibility to autoimmune disease, are examined.—Trop. Dis. Bull. **93** (1996) 1025

**Buchwalow, I. B., Brich, M. and Kaufmann, S. H. E.** Signal transduction and phagosome biogenesis in human macrophages during phagocytosis of *Mycobacterium bovis* BCG. Acta Histochem. **99** (1997) 63–70.

Downstream signal transduction via heterotrimeric GTP-binding proteins to protein kinase C (PKC) has been reported to be a central event in induction of rapid phagocytosis of extracellular particles by macrophages. However, the signaling pathway involved in mycobacterial uptake and phagosome biogenesis is poorly understood, and there is lack of information about *in situ* localization of PKC, cytoskeletal proteins, and G-proteins in mycobacterial vacuoles. Employing immunocytochemical methods, we provide evidence that alpha-subunits of stimulatory and inhibitory G-proteins and PKC beta as well as two major cytoskęletal components, microfilaments and microtubules, participate in uptake of *Mycobacterium bovis* BCG by human macrophages and co-localize in phagosomes. This implies that cellular signaling via G-proteins and PKC beta may occur not only at the level of the plasma membrane; rather, the alpha-subunit of G-proteins and PKC beta may be translocated to the effector proteins involved in phagosomal biogenesis. A similar pattern of accumulation of G-proteins, PKC, and both microfilamental and microtubular cytoskeleton around vacuoles containing internalized latex beads indicates their general role in phagocytosis.—Authors' Abstract

**Cooper, A. M., D'Souza, C., Frank, A. A. and Orme, I. M.** The course of *Mycobacterium tuberculosis* infection in the lungs of mice lacking expression of either perforin- or granzyme-mediated cytolytic mechanisms. Infect. Immun. **65** (1997) 1317–1320.

CD8 T cells have been shown to be protective against *Mycobacterium tuberculosis* infections in the mouse. These cells have been shown to be cytolytic toward *M. tuberculosis*-infected cells and have also been shown to release the protective cytokine gamma interferon in response to mycobacterial antigen. It has therefore been unclear how these cells mediate their protective response. To dissect this problem, we compared the courses of *M. tuberculosis* infections in control, perforin gene-knockout, and granzyme gene-knockout mice exposed by the realistic pulmonary route. The inability to express either of these molecules limits the expression of the major lytic pathway but does not appear to influence the course of the infection or result in any discernible histologic differences. These data seem to rule against a lytic role for CD8 T cells in the lungs and, hence, tend to suggest instead that another type of mechanism, such as cytokine secretion by these cells, is their primary mode of action.—Authors' Abstract

**Denis, O., Lozes, E. and Huygen, K.** Induction of cytotoxic T-cell responses against culture filtrate antigens in *Mycobacterium bovis* bacillus Calmette-Guerin-infected mice. Infect. Immun. **65** (1997) 676–684.

CD8+ T cells are essential for protection against mycobacteria, as is clearly demonstrated by the fatal outcome of experimental infection of beta-2 microglobulin knockout mice. However, the mechanisms and antigens (Ags) leading to CD8+ T-cell activation and regulation have been poorly characterized. Here we show that, upon immunization of major histocompatibility complex (MHC)-congenic mice with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG), a cytotoxic response against BCG culture filtrate (CF) Ags (CFAgs) is induced in H-2 (b) and H-2 (bxd) haplotypes but not in the H-2 (d) haplotype. This response is mediated by CD8+ T cells and absolutely requires the activation of CD4+ T cells and their secretion of interleukin 2. The lack of cytotoxic response in H-2 (d) mice cannot be explained by impaired cytokine production or by a defect in Ag presentation by H-2 (d) macrophages. Using the MHC class I mutant B6.C-H-2 (bm13) mouse strain, we demonstrate that cytotoxic T lymphocytes

(CTLs) recognize CFAgs exclusively in association with D-b molecules. These Ags are crossreactive in mycobacteria, since BCG-induced CTLs also recognize macrophages pulsed with CF from *M. tuberculosis* H37Rv and H37Ra and from two virulent strains of *M. bovis*. Moreover, immunization with *M. kansasii* induces CTLs able to lyse macrophages pulsed with BCG CF. Finally, we have found that these Ags can be characterized as hydrophilic proteins, since they do not bind to phenyl-Sepharose CL-IB. Our results indicate that MHC-linked genes exert a profound influence on the generation of CD8+ CTLs following BCG vaccination.—Authors' Abstract

**D'Souza, C. D., Cooper, A. M., Frank, A. A., Mazzaccaro, R. J., Bloom, B. R. and Orme, I. M.** An anti-inflammatory role for gamma delta T lymphocytes in acquired immunity to *Mycobacterium tuberculosis*. J. Immunol. **158** (1997) 1217–1221.

Although a role for gamma delta receptor-bearing T cells in the acquired immune response to infection with *Mycobacterium tuberculosis* is suggested by several lines of evidence, the only data indicating a possible role in specific protective immunity have been provided by very high dose i.v. infection models. In the current study, more modest low-dose inocula delivered by the aerosol route grew identically in wild-type controls and in mutant mice in which the CS gene of the gamma delta TCR has been disrupted by homologous recombination. This situation did not change if the inoculum size was increased or if an aerosol challenge with a *M. tuberculosis* strain of higher virulence was given. However, while the control and containment of these infections was similar, the mutant mice exhibited a substantial pyogenic form of the granulomatous response compared with the lymphocytic response seen in control animals, a finding that may well explain mortality in the former group if high i.v. doses are given. These data indicate that gamma delta T cells do not directly contribute to protection against tuberculosis or that they do so only when bacterial loads are very high. Instead, the data suggest that gamma delta T cells perhaps play an important role by influencing local cellular traffic, promoting the influx of lymphocytes and monocytes, and limiting the access of inflammatory cells that do not contribute to protection but may cause damage.—Authors' Abstract

**Fink, S., Finiasz, M. R., Valdez, R., dela Barrera, S. and Sasiain, M. D.** [Evaluation of cytokine production in leprosy patients.] Medicina Buenos Aires **56** (1996) 705–708. (in Spanish)

The aim of the present study was to evaluate the cytokine production by peripheral blood mononuclear cells (PBMC) of leprosy patients when the cells were stimulated in culture by ConA, PPD or *M. leprae*. We measured IL-2, IL-4, IFN-gamma and IL-6 in cell-free supernatants by enzyme-linked immunoassays. Our results do not suggest a clear association of a clinical form of leprosy with either Th1 or Th2 cytokine secretion profile in PBMC of leprosy patients.—Authors' English Abstract

**Gao, J., *et al.*** [On SIL-2R in the sera of persons cured of leprosy.] China Lepr. J. **12** (1996) 238–240. (in Chinese)

Soluble interleukin-2 receptor (SIL-2R) was determined in 58 persons cured of leprosy and 27 members of their families in Lanxi Prefecture, Zhejiang, China, taking 28 local healthy residents as controls. The results showed that the level of SIL-2R in persons who had MB leprosy, especially LL, was lower than in those who had PB leprosy and controls ($p < 0.002$), and had nothing to do with the disease duration and time to have been cured. The contents of SIL-2R in the members of the ex-patients' families were the same as in the controls ($p > 0.05$).—Authors' English Abstract

**Geluk, A., van Meijgaarden, K. E., de Vries, R. R. P., Sette, A. and Ottenhoff, T. H. M.** A DR17-restricted T cell epitope from a secreted *Mycobacterium tuberculosis* antigen only binds to DR17 molecules at neutral pH. Eur. J. Immunol. **27** (1997) 842–847.

The assembly of peptide-major histocompatibility class II complexes *in vitro* is accelerated at low pH, comparable to that found in the intracellular compartments of metabolically active antigen-presenting cells (APC). Mycobacteria such as *Mycobacterium tuberculosis* reside in phagosomes with only mildly acidic pH. Therefore, we investigated the pH dependency of peptide-HLA-DR binding for several T-cell epitopes of mycobacterial proteins, focusing particularly on well-defined, immunodominant HLA-DR17(3)-restricted T-cell epitopes: peptide (p) 3–13 from the cytoplasmic 65-kDa heat shock protein of *M. tuberculosis/M. leprae*, and peptide 56–65 from the secreted 30/31-kDa protein from *M. tuberculosis/M. leprae*. P3–13 bound to purified, cell-free DR17 under both acidic and neutral conditions. Four other, unrelated DR17-binding peptides showed the same pH-dependent binding characteristics as p3–13. P56–65, however, only bound to purified DR17 at pH 7 but not at all at pH 4.5. These DR17 peptide binding data were confirmed in cell-bound DR17, in T-cell stimulation assays in which fixed APC were peptide-pulsed at acidic or neutral pH before addition of peptide-specific DR17-restricted T cells. As far as we are aware, p56–65 is the only human T-cell epitope binding to HLA exclusively at neutral pH. The binding characteristics of p56–65 may reflect dominant processing in alternative, less acidic vacuolar compartments specifically related to the generation of epitopes from (secreted) mycobacterial proteins. The observation that p56–65 is an immunodominant epitope for anti-mycobacterial T cells suggests the relevance of such novel processing compartments in T-cell-mediated immunity.—Authors' English Abstract

**Ishaque, M., Sticht Groh, V. and Togola, D.** Susceptibility of severe combined immunodeficient mice to *Mycobacterium leprae*. Microbios **88** (1996) 19–26.

The susceptibility of severe combined immunodeficient (SCID) mice to human leprosy bacilli was investigated. Nude mice were used as controls. SCID mice were found to be highly susceptible to *Mycobacterium leprae* and the progress of infection was comparatively 2–3 months earlier than observed in the nude mice. After reaching a maximum of similar to $1 \times 10^9$ acid-fast bacilli/foot pad at about 8 months postinfection, the number of bacilli gradually decreased. The progress of infection in nude mice was different from that found in SCID mice. The multiplication of *M. leprae* in the foot pads of nude mice continued and reached similar to $2.0 \times 10^{10}$ bacilli/foot pad and then nearly remained the same. The results indicate that SCID mice can be used as a suitable model for screening antileprosy drugs while nude mice should be involved in the production of *M. leprae* for use in other fields of leprosy research.—Authors' Abstract

**Khare, S., Bhutani, L. K. and Rao, D. N.** Release of reactive nitrogen intermediates from the peripheral blood-derived monocytes/macrophages of leprosy patients stimulated *in vitro* by tuftsin. Lepr. Rev. **68** (1997) 16–24.

The production of reactive nitrogen intermediates (RNI) by macrophages is critical to host defense, particularly for exerting the bactericidal and tumoricidal properties. Nitric oxide (NO) was measured in the peripheral blood-derived monocytes/macrophages of normal and leprosy patients (BT/TT and BL/LL) in the presence and absence of "tuftsin" as a function of *in vitro* culture age (on 1, 3, 7 days). Macrophages from both groups of leprosy patients were able to produce NO during the unstimulated state but only BL/LL macrophages could be activated by tuftsin to produce significantly high levels of NO. This increase was highest on day 1, then gradually decreased with *in vitro* culture age. Surprisingly, tuftsin was unable to enhance the NO production in normal macrophages above the basal level. Further, normal and BT/TT macrophages had only Cu-Zn derived superoxide dismutase (SOD) activity, whereas BL/LL cultures had Cu-Zn and Mn derived SOD activity. These studies indicate that in BL/LL cultures: a) apart from tuftsin, some additional signal is required to activate nitric oxide synthase (NOS) gene for NO production; and b) Mn-SOD produced by *Mycobacterium leprae* is playing a defensive

role against toxic-free radicals. The final outcome of this mechanism is the survival of *M. leprae* inside the macrophages.—Authors' Summary

**Libraty, D. H., Airan, L. E., Uyemura, K., Jullien D., Spellberg, B., Rea, T. H. and Modlin, R. L.** Interferon-gamma differentially regulates interleukin-12 and interleukin-10 production in leprosy. J. Clin. Invest. **99** (1997) 336–341.

The ability of monocytes to influence the nature of the T-cell response to microbial pathogens is mediated in part by the release of cytokines. Of particular importance is the release of IL-12 and IL-10 by cells of the monocyte/macrophage lineage upon encountering the infectious agent. IL-12 promotes cell-mediated immunity (CMI) to intracellular pathogens by augmenting T-helper type I responses, whereas IL-10 downregulates these responses. The ability of gamma interferon (IFN-$\gamma$) to modulate the balance between IL-12 and IL-10 production was examined by studying leprosy as a model. In response to *Mycobacterium leprae* stimulation, IFN-$\gamma$differentially regulated IL-12 and IL-10 production resulting in upregulation of IL-12 release and downregulation of IL-10 release. Furthermore, we determined that the mechanism by which IFN-$\gamma$ downregulates IL-10 was through the induction of IL-12. The data suggest a model of lymphocyte-monocyte interaction whereby the relative presence or absence of IFN-$\gamma$in the local microenvironment is a key determinant of the type of monocyte cytokine response, and hence the degree of CMI in the host response to infection.—Authors' Abstract

**Mitra, D. K., Rajalingam, R., Taneja, V., Bhattacharya, B. C. and Mehra, N. K.** HLA-DR polymorphism modulates the cytokine profile of *Mycobacterium leprae* HSP-reactive CD4+ T cells. Clin. Immunol. Immunopathol. **82** (1997) 60–67.

In the present study, *in vitro* attempts have been made to define the cytokine profile of CD4+ T cells from polar leprosy patients and healthy individuals against *Mycobacterium leprae*-derived heat shock proteins (hsp), hsp65 and hsp18, and their trypsin-digested fragments, relating to HLA-DR polymorphism. While all tryptic fragments of optimal digestion and undigested hsp could stimulate CD4+ T cells from tuberculoid (TT) leprosy patients and healthy contacts (stimulation index, SI > 2.0), only two fragments, TDB65–2 (18 kDa) and TDB18–3 (3 kDa)+ triggered CD4+ T cells of anergic lepromatous (LL) leprosy patients. Both of these hsp and their tryptic fragments showed diverse HLA-DR restriction, with DR15 providing the strongest restriction. Cytokine analysis demonstrated that hsp65 and hsp18 induced Th1-like activity in the context of all the restricting HLA-DR alleles, except DR1 and DR7 which induced a Th2-type of response against hsp65 and hsp18, respectively. These Th2 inducer epitopes on hsp65 (DR1 restricted) and hsp18 (DR7 restricted) were absent from TDB65–2 and TDB18–3 which exclusively triggered Th1 cells in both TT and LL forms of leprosy in the context of multiple DR alleles, DR15 being the major antigen-presenting allele. These studies suggest that the major histocompatibility complex phenotype of the antigen-presenting cell can modulate Th1-like versus Th2-like activity against *M. leprae* pathogens in leprosy and healthy individuals.—Authors' Abstract

**Nakanga, K., Nomaguchi, H. and Matsuoka, M.** [Establishment of mouse cell lines expressing *Mycobacterium leprae* 65kDa heat shock protein gene.] Jpn. J. Lepr. **65** (1996) 113–120. (in Japanese)

The authors established mouse cell lines (BALB/3T3 clone A31–1-1) that showed stable expression of *Mycobacterium leprae* 65 kDa heat-shock protein (hsp65), demonstrated by both protein and mRNA analysis. They believe that such cell lines would work as target cells in cytolysis assays *in vitro* used to study T lymphocytes generated during *M. leprae* infection.—Trop. Dis. Bull. **94** (1997) 128

**Nomaguchi, H., Fututomi, Y., Nagai, S., Yogi, Y., Okamura, H., Ohara, N., Matsuoka, M., Nagata, K. and Yamada, T.** [BCG vaccination to *Mycobac-*

*terium leprae* infection in mice.] Jpn. J. Lepr. **65** (1996) 106–112. (in Japanese)

BCG vaccine (Tokyo strain) was given in BALB/cA mice intradermally 1 or 3 months before *Mycobacterium leprae* challenge as modified Shepard's method. The vaccine dosage was $10^{7-8}$ or $10^{6}$. The vaccine gave good protection in both dosages and both challenges against *M. leprae* infection. Lymphocyte proliferation of BCG-vaccinated splenocyte cultures in response to *M. leprae* lysate or BCG components (heat shock protein (hsp)65, 38 kDa, 30 kDa or 12 kDa protein) were tested, and potent proliferative responses were seen in the cultures with *M. leprae* lysate and hsp65. Furthermore, $\gamma$-interferon (IFN) productions were positive in the cultures with *M. leprae* lysate or hsp65, but negative with other antigens. The production of $\gamma$-IFN with hsp65 was never inhibited with polymyxin B but was inhibited with interleukin-10. The authors conclude that BCG (Tokyo strain) is a useful vaccine for *M. leprae* infection in mice, and one of the components of BCG, hsp65, may be an effective antigen component for protection of *M. leprae* infection inducing Th1-type cytokine.—Trop. Dis. Bull. **94** (1997) 129–130

**Parkash, O. M., Beuria, M. K., Girdhar, B. K., Katoch, K. and Sengupta, U.** Efforts in diagnosing early leprosy using serological techniques. J. Biosci. **22** (1997) 111–116.

Skin scrapings obtained from the lesions of leprosy patients of all types showed 96% positivity to the serum antibody competition test using monoclonal antibody (ML04) to 35-kDa antigen of *Mycobacterium leprae*. Further, *in vitro* culture of full thickness skin biopsies from lepromatous patients were noted to release IgG antibodies to *M. leprae* with a peak antibody response at 48 hr. The significance of this local antibody response to *M. leprae* in skin has been discussed for its possible use in diagnosing early leprosy.—Authors' Abstract

**Pimentel, M. I. F., Sampaio, E. P., Nery, J. A. C., Gallo, M. E. N., Saad, M. H. F., Machado, A. M., Duppre, N. C. and Sarno, E. N.** Borderline-tuberculoid leprosy: clinical and immunological heterogeneity. Lepr. Rev. **67** (1996) 287–296.

The authors analyzed some immunological criteria in leprosy patients diagnosed as borderline tuberculoid by the presentation of different grades of skin lesions as well as different grades of nerve involvement. Only 50% of the patients presented a single skin lesion and 58% had none or only one affected nerve. Nineteen patients (39.6%) showed a positive lepromin reaction (induration $\geq 5$ mm).

Patients with a positive skin test had a greater number of skin lesions when compared with patients with a negative lepromin test. Fifty-seven percent of the patients were found to be positive using a lymphoproliferation test (LTT) in response to *Mycobacterium leprae* antigens. Positive LTT results did not correlate with the number of skin lesions, but patients unresponsive to LTT had a lesser extent of nerve involvement. Four out of 18 patients (22%) released high IFN$\gamma$ levels in PBMC culture stimulated by *M. leprae* (mean U/ml $\pm$ SD $= 142 \pm 72$). All of these four patients presented only one skin lesion, although three of them had more than one affected nerve.

Nineteen out of 21 patients (90.5%) showed no anti-PGL-I antibodies in their serum. The low levels of anti-PGL-I antibodies among these patients confirmed their tuberculoid background even in those with multiple skin lesions.

These findings seem to attribute an important role to IFN$\gamma$ in restraining the spreading of the infection in the skin, but IFN$\gamma$ may have an opposite effect on the nerves. The potential pathological effects of IFN$\gamma$ during the delayed type of hypersensitivity can be related to its ability to synergize with other inflammatory cytokines such as TNF$\alpha$, IL-1$\beta$, and others.—Authors' Summary

**Shannon, E. J., Morales, M. F. and Sandoval, F.** Immunomodulatory assays to study structure-activity relationships of thalidomide. Immunopharmacology **35** (1997) 203–212.

Thalidomide, which has a long history of tragedy because of its ability to cause se-

vere birth defects, is very effective in alleviating erythema nodosum leprosum in leprosy patients and aphthous ulcers in AIDS patients. The causes of these inflammatory diseases and the mechanism by which thalidomide diminishes them are unknown. It has been suggested that modulation of the immune response plays an important role. We found that thalidomide exerts immunomodulatory activity in three bioassays. It suppresses an IgM plaque-forming cell response in mice injected with sheep erythrocytes; it inhibits tumor necrosis factor-alpha (TNF-$\alpha$) production by LPS-stimulated human mononuclear cells; and it enhances IL-2 production by Con-A-stimulated human mononuclear cells. We employed these bioassays to compare the activity of 15 analogs of thalidomide with thalidomide itself. Eight of the compounds were derivatives of the glutarimide moiety of thalidomide and the others were phthalimide or derivatives of the phthalimide moiety of thalidomide. N-hydroxyphthalimide, a simple derivative of phthalimide, was more effective than thalidomide and was also the most effective of the compounds assayed in suppressing the IgM plaque and TNF-$\alpha$ response; but it did not enhance the IL-2 response; instead, it significantly suppressed it.—Authors' Abstract

**Shannon, E. J., Sandoval, F. and Krahenbuhl, J. L.** Hydrolysis of thalidomide abrogates its ability to enhance mononuclear cell synthesis of IL-2 as well as its ability to suppress the synthesis of TNF-alpha. Immunopharmacology **36** (1997) 9–15.

Thalidomide is effective in the treatment of inflammatory conditions like erythema nodosum leprosum in leprosy patients, and aphthous ulcers in AIDS patients. Its mechanism of action is uncertain and reports of its effect on the synthesis of inflammatory cytokines such as IL-2 and tumor necrosis factor-alpha (TNF-$\alpha$) are contradictory. As thalidomide is labile to spontaneous hydrolysis at pH 7.4, studies were carried out to explore the effects of deliberate hydrolysis on the ability of thalidomide to modulated cytokine production by human mononuclear cells stimulated *in vitro* with *staphylococcal* enterotoxin A (SEA) (IL-2) or lipopolysaccharide from *Salmonella minnesota* (LPS) (TNF-$\alpha$). Unhydrolyzed thalidomide at 4.0 $\mu$g/ml consistently enhanced the synthesis of IL-2 in SEA-stimulated cells, and suppressed the synthesis of TNF-$\alpha$ in LPS-stimulated cells; whereas, hydrolyzed thalidomide had no enhancing effect on SEA-stimulated cell synthesis of IL-2 or suppressive effect on LPS-stimulated cell synthesis of TNF-$\alpha$. These findings demonstrate that thalidomide's ability *in vitro* to enhance IL-2 and to suppress TNF-$\alpha$ in stimulated cells is dependent on the intact molecule and underscore the necessity to employ thalidomide under appropriate physicochemical conditions.—Authors' Abstract

**Sugita, Y., Miyamoto, M., Koseki, M., Ishii, N. and Nakajima, H.** Suppression of tumour necrosis factor-alpha expression in leprosy skin lesions during treatment for leprosy. Br. J. Dermatol. **136** (1997) 393–397.

The expression of tumor necrosis factor-alpha (TNF-$\alpha$) in leprosy skin lesions was examined before and during successful treatment in a patient with borderline lepromatous leprosy. Before treatment, immunohistochemical staining of a skin-biopsy specimen showed diffuse TNF-$\alpha$ deposits in granulomas and significant TNF-$\alpha$ deposits on infiltrated mononuclear cells. After 1 year's treatment, the skin lesions exhibited a reduction in granulomas, and a concomitant reduction in deposits of TNF-$\alpha$. Furthermore, the level of expression of TNF-$\alpha$ messenger RNA, as examined using a reverse transcriptase-polymerase chain reaction method, was reduced markedly after treatment. These findings provide evidence for a correlation between the expression of TNF-$\alpha$ and disease activity, suggesting that TNF-$\alpha$ is a useful prognostic indicator for inflammation in leprosy.—Authors' Abstract

**Supek, F., Supekova, L., Nelson, H. and Nelson, N.** Function of metal-ion homeostasis in the cell division cycle, mitochondrial protein processing, sensitivity to mycobacterial infection and brain function. J. Exp. Biol. **200** (1997) 321–330.

A novel *Saccharomyces cerevisiae* mutant, unable to grow in the presence of 12.5 mmol $l^{-1}$ EGTA, was isolated. The phenotype of the mutant is caused by a single amino acid change (Gly149 to Arg) in the essential yeast cell division cycle gene CDC1. The mutant could be suppressed by overexpression of the SMF1 gene, which codes for a plasma membrane Mn2+ transporter. We observed that the yeast SMF1 gene shares homology with the mouse Nramp gene. Nramp (Bcg) was cloned as a gene responsible for mouse resistance to infection with mycobacteria and is identical with the Ity and the Lsh genes conferring resistance to infection by *Salmonella typhimurium* and *Leishmania donovani*, respectively. Although the cloning of Nramp identified the gene responsible for the resistance of mice to mycobacteria, its function is unknown. We propose that the mammalian protein, like the yeast transporter, is a Mn2+ and/or Zn2+ transporter. Following the phagocytosis of a parasite into the phagosome, the macrophage produces reactive oxygen and/or nitrogen intermediates that are toxic for the internalized bacteria. The survival of the pathogen during the burst of macrophage respiratory activity is thought to be partly mediated by microbial superoxide dismutase (SOD), which contains Mn2+ or Fe2+ in its active center. Nramp may transport Mn-2+ from the extracellular milieu into the cytoplasm of a macrophage and, after the generation of the phagosome, remove Mn2+ from the organelle. Thus, the Mn2+-depletion of the phagosome microenvironment by the Nramp gene product may be a rate-limiting step in the metalloenzyme's production by the engulfed bacteria. This limitation will restrict the mycobacterial ability to produce active enzymes such as SOD and prevent the propagation of the ingested microorganisms. Conversely, an increased concentration of Mn2+ in the phagosome caused by a defective Nramp transporter ($Bcg^s$) may promote the growth of the mycobacteria and render the organism sensitive to the pathogen. We use a similar approach to identify, clone and study other metal-ion transporters.—Authors' Abstract

**Vallishayee, R. S., Gupte, M. D., Anantharaman, D. S. and Nagaraju, B.** Post-vaccination sensitization with ICRC vaccine. Indian J. Lepr. **68** (1996) 167–174.

ICRC vaccine is one of the candidate antileprosy vaccines under test in a large-scale comparative vaccine trial in India. The objective of the present study (done in July–December 1992) was to assess the sensitization potential and reactogenicity of this vaccine preparation in the local population in the trial area (in Tamil Nadu). The study included 368 "healthy" individuals aged 1–70 years. Each individual received either ICRC vaccine or normal saline (control) by random allocation. They were also tested with Rees' MLSA (*Mycobacterium leprae* soluble antigen) and lepromin-A 12 weeks after vaccination. Reactions to MLSA were measured after 48 hours and those to lepromin-A after 48 hours and 3 weeks. The character and size of the local response, at the vaccination site, were recorded at the 3rd, 8th and 15th week after vaccination. Healing of the vaccination lesion was uneventful, the mean size of the lesion being 10.3 mm. The mean sizes of postvaccination reactions to MLSA and lepromin (both early and late reactions) were significantly higher in the vaccine group compared with those in the normal saline group; the sensitizing effect attributable to the vaccine was of the order of 3.5 mm, 1.7 mm and 2.2 mm, respectively. The authors concluded that the ICRC vaccine was "safe" and produced a significant sensitizing effect, as measured by postvaccination sensitization to MLSA and lepromin, in the local population.—Trop. Dis. Bull. **93** (1996) 1041

**Visentainer, J. E. L., Tsuneto, L. T., Serra, M. F., Peixoto, P. R. F. and Petzl Erler, M. L.** Association of leprosy with HLA-DR2 in a southern Brazilian population. Braz. J. Med. Biol. Res. **30** (1997) 51–59.

The association between HLA specificities and leprosy was investigated in a Southern Brazilian population. One hundred-twenty-one patients and 147 controls were typed for HLA-A, B, Cw, DR and DQ. Patients were subdivided into the following subgroups, according to clinical, histological and immunological criteria:

lepromatous (N = 55), tuberculoid (N = 32), dimorphous (N = 20), and indeterminate (N = 14). The frequencies of HLA specificities were compared between the total group of patients and controls, and between the same controls and each subgroup of patients. After correction of the probabilities, deviations were not significant, except for the DR2 specificity, which presented a frequency of 44.2% in the total group of patients and 56.3% in the subgroup of individuals with the tuberculoid form of the disease, compared to 23.3% in the controls. Stratified analysis showed that the increased DR2 frequency in the total group of patients was due to the subgroups with the tuberculoid and dimorphous forms. The relative risk of tuberculoid leprosy for DR2-positive individuals was 4.2, and the etiologic fraction of DR2 was 0.429. In conclusion, a positive association of the DR2 specificity with the tuberculoid form of leprosy, but not with the lepromatous, dimorphous, or indeterminate forms, was demonstrated in this Southern Brazilian population.—Authors' Abstract

**Weng, X., Zhang, C., Chen, S., *et al.*** [Detection of PGL-I antigen and S100 protein in the histopathologic diagnosis of leprosy.] Chin. J. Clin. Dermatol. **25** (1996) 270–272. (in Chinese)

Immunohistopathologic staining technique using monoclonal antibody against PGL-I antigen and anti-S100 protein antibody were applied along with the routine histopathologic HE and AF staining techniques on 9 confirmed patients with paucibacillary (PB) and 1 confirmed multibacillary (MB) leprosy as well as on 1 suspected case of PB and 11 MB clinically suspected relapsed cases of leprosy. In the 9 confirmed PB patients, 3 were AF, 7 PGL-I and 6 S100 protein staining positive; but in the suspected PB relapsed case, although AF and PGL-I staining were negative, S100 protein staining was positive in the infiltrated areas of the biopsy. In the confirmed MB cases all the specific criteria were positive; whereas in the 11 suspected MB relapsed cases only 8 showed AF and PGL-I staining positive, 6 showed S100 protein staining positive. In the 3 AF and PGL-I staining negative cases, relapse cannot be confirmed. Thus PGL-I and S100 protein staining are of assistance in the diagnosis of early leprosy and in the verification of relapse from reversal reaction in problem cases.—Authors' English Abstract

**Wesch, D., Marx, S. and Kabelitz, D.** Comparative analysis of alpha beta and gamma delta T cell activation by *Mycobacterium tuberculosis* and isopentenyl pyrophosphate. Eur. J. Immunol. **27** (1997) 952–956.

Phosphorylated nonpeptide compounds have recently been identified as potent mycobacteria-derived ligands for human V gamma 9/V delta 2-expressing gamma delta T cells.

Crude mycobacterial extracts also contain protein antigens which stimulate CD4 alpha beta T cells to produce growth factors that are used by gamma delta T cells for clonal expansion. We have investigated the dynamics *in vitro* of expansion of CD4 T cells and V gamma 9 cells in cultures of peripheral blood mononuclear cells stimulated with synthetic isopentenyl pyrophosphate (IPP) in the absence or presence of additional stimuli. The results indicated that following stimulation with IPP, gamma delta T cells express CD25 and CD69 antigens, but fail to proliferate unless growth factors are provided exogenously or endogenously through activation of CD4 T cells by additional stimuli, such as tetanus toxoid, alloantigen, or superantigens. Furthermore, the presence of antigen presenting cells are required for expansion of gamma delta T cells. In response to IPP stimulation, purified CD4 T cells neither express CD25 or CD69, nor do they proliferate even in the presence of exogenous IL-2. Apart from IL-2, IL-15 and, less efficiently, IL-4, IL-7, and IL-12 can contribute to cellular expansion of IPP-reactive V gamma 9 cells. Together, the results demonstrate that peripheral blood gamma delta T cells proliferate in response to IPP only if CD4 T cells are simultaneously activated by an additional stimulus. This mechanism provides a tight control of the reactivity of gamma delta T cells towards phosphorylated nonpeptide antigens.—Authors' Abstract

# Microbiology

**Ainsa, J. A., Perez, E., Pelicic, V., Berthet, F.-X., Gicquel, B. and Martin, C.** Aminoglycoside 2′-*N*-acetyltransferase genes are universally present in mycobacteria: characterization of the *aac(2′)-Ic* gene from *Mycobacterium tuberculosis* and the *aac(2′)-Id* gene from *Mycobacterium smegmatis*. Mol. Microbiol. **24** (1997) 431–441.

The genus *Mycobacterium* comprises clinically important pathogens such as *Mycobacterium tuberculosis*, which has reemerged as a major cause of morbidity and mortality worldwide especially with the emergence of multidrug-resistant strains. The use of fast-growing species such as *M. smegmatis* has allowed important advances to be made in the field of mycobacterial genetics and in the study of the mechanisms of resistance in mycobacteria. The isolation of an aminoglycoside-resistance gene from *M. fortuitum* has recently been described. The *aac(2′)-Ib* gene is chromosomally encoded and is present in all isolates of *M. fortuitum*. The presence of this gene In other mycobacterial species is studied here and genes homologous to that of *M. fortuitum* have been found in all mycobacterial species studied. In this report, the cloning of the *aac(2′)-Ic* gene from *M. tuberculosis* H37Rv and the *aac(2′)-Id* gene from *M. smegmatis* mc²155 is described. Southern blot hybridizations have shown that both genes are present in all strains of this species studied to date. In addition, the putative *aac(2′)-Ie* gene has been located in a recent release of the *M. leprae* genome. The expression of the *aac(2′)-Ic* and *aac(2′)-Id* genes has been studied in *M. smegmatis* and only *aac(2′)-Id* is correlated with aminoglycoside resistance. In order to elucidate the role of the aminoglycoside 2′-*N*-acetyltransferase genes in mycobacteria and to determine whether they are silent resistance genes or whether they have a secondary role in mycobacterial metabolism, the *aac(2′)-Id* gene from *M. smegmatis* has been disrupted in the chromosome of *M. smegmatis* mc²155. The disruptant shows an increase in aminoglycoside susceptibility along with a slight increase in the susceptibility to lysozyme.—Authors' Summary

**Dhandayuthapani, S., Mudd, M. and Deretic, V.** Interactions of OxyR with the promoter region of the oxyR and ahpC genes from *Mycobacterium leprae* and *Mycobacterium tuberculosis*. J. Bacteriol. **179** (1997) 2401–2409.

In contrast to the intact oxyR gene (a homolog of the central regulator of peroxide stress response in enteric bacteria) in *Mycobacterium leprae*, this gene is inactive in all strains of *M. tuberculosis*. In both species, oxyR is divergently transcribed from ahpC, which encodes a homolog of alkyl hydroperoxide reductase. To initiate investigations of the regulation of oxidative stress in mycobacteria and consequences of the elimination of oxyR in *M. tuberculosis*, in this work we tested the hypothesis that mycobacterial OxyR acts as a DNA binding protein and analyzed its interactions with the oxyR and ahpC promoters. *M. leprae* OxyR was overproduced and purified, and its binding to the oxyR-ahpC intergenic region of *M. leprae* was demonstrated. By using a sequential series of overlapping DNA fragments, the minimal OxyR binding site was delimited to a 30-bp DNA segment which included a palindromic sequence conforming with the established rules for the LysR family of regulators. A consensus sequence for the mycobacterial OxyR recognition site (cTTATCggc-N-3-gccGATAAg) was deduced based on its conservation in different mycobacteria. A variance in two potentially critical nucleotides within this site was observed in *M. tuberculosis*, in keeping with its reduced affinity for OxyR. Transcription of plasmid-borne *M. leprae* oxyR and ahpC was investigated in *M. smegmatis* and *M. bovis* BCG by S1 nuclease protection and transcriptional fusion analyses. Two mRNA 5′ ends were detected in each direction: (i) P (1) oxyR and P (2) oxyR and (ii) P (1) ahpC and P (2) ahpC. The binding site for OxyR overlapped P (1) oxyR, reminiscent of the autoregulatory loops controlling expression of oxyR in en-

teric bacteria and characteristic of the LysR superfamily in general. This site was also centered 65 bp upstream of P (1) ahpC, matching the usual position of LysR-type recognition sequences in relationship to positively controlled promoters. Superimposed on these features was the less orthodox presence of multiple transcripts and their unique arrangement, including a region of complementarity at the 5′ ends of the P (2) ahpC and P (2) oxyR mRNAs, suggesting the existence of complex regulatory relationships controlling oxyR and ahpC expression in mycobacteria.—Authors' Abstract

**Eiglmeier, K., Fsihi, H., Heym, B. and Cole, S. T.** On the catalase-peroxidase gene, katG, of *Mycobacterium leprae* and the implications for treatment of leprosy with isoniazid. FEMS Microbiol. Lett. **149** (1997) 273–278.

The toxicity of the potent tuberculocidal agent, isoniazid, is mediated by the heme-containing enzyme, catalase-peroxidase, encoded by the katG gene. Although isoniazid has been used for the treatment of leprosy, it is shown here that the katG gene of *Mycobacterium leprae* is a pseudogene, which has probably been inactivated by multiple mutations. Inactive genes were detected by the polymerase chain reaction in several isolates of *M. leprae* of different geographical origins, and attempts to complement an isoniazid-resistant strain of *M. smegmatis* with the katG pseudogene were unsuccessful. Isoniazid is thus likely to be of no therapeutic benefit to leprosy patients.—Authors' Abstract

**Esaguy, N. and Aguas, A. P.** Subcellular localization of the 65-kDa heat shock protein in mycobacteria by immunoblotting and immunogold ultracytochemistry. J. Submicroscop. Cytol. Pathol. **29** (1997) 85–90.

The 65-kDa heat shock protein (hsp65) is an immunodominant antigen in mycobacterial infections and also the key etiologic factor in mycobacteria-induced autoimmune arthritis. Because the subcellular distribution of hsp65 in the mycobacteria may be relevant to understand its immunoreactivity, we have investigated the presence of hsp65 in the envelope and cytoplasmic compartments of the bacilli. Anti-hsp65 antibodies were used in Western blottings to investigate the presence of hsp65 in cell fractions (membrane, envelope and cytosol) of *Mycobacterium avium* and *M. smegmatis*, and also to label hsp65 *in situ* by the immunogold method on thin-sectioned mycobacteria, including the noncultivable *M. leprae* that were studied by transmission electron microscopy. All of the three subcellular mycobacterial fractions showed significant labeling by anti-hsp65 antibodies. Immunogold ultracytochemistry revealed the presence of hsp65 in both the cytoplasm and the envelope of mycobacteria. The data indicate that hsp65 molecules are commonly present not only in the cytoplasm but also in the envelope of mycobacteria. The latter topography of hsp65 may contribute to the strong immunogenicity of hsp65 since it may correspond to export hsp65 molecules captured before being secreted into the extracellular milieu, thus making hsp65 a mycobacterial antigen readily available for presentation to the immune system of infected hosts.—Authors' Abstract

**Han, M. Y., Son, M. Y., Lee, S. H., Kim, J. K., Huh, J. S., Kim, J. H., Choe, I. S., Chung, T. W. and Choe, Y. K.** Molecular cloning of the leuB genes from *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis*. Biochem. Mol. Biol. Int. **41** (1997) 657–663.

A gene responsible for the biosynthesis of leucine has been cloned by the complementation of the *Escherichia coli* leuB6 auxotroph mutant after transformation with the *Mycobacterium bovis* BCG genomic DNA library, which was constructed by ligating the partially digested BCG DNA with Sau3AI into the pUC19 digested with BamHI. Sequencing of the leuB gene of BCG revealed an ORF (open reading frame) of 1011 bp encoding isopropylmalate dehydrogenase with a calculated molecular weight of 42 kDa. The leuB gene of *M. tuberculosis* isolated from a Korean tuberculosis patient is shown to be identical to that of BCG except one bp.—Authors' Abstract

**Jou, N. T., Yoshimori, R. B., Mason, G. R., Louie, J. S. and Liebling, M. R.** Single-tube, nested reverse transcriptase PCR for detection of viable *Mycobacterium tuberculosis*. J. Clin. Microbiol. **35** (1997) 1161–1165.

Several problems remain before molecular biology-based techniques, such as PCR, are widely accepted for the detection of infectious agents. Among the most formidable of these problems are the inability of the tests to distinguish between viable and nonviable organisms. We approached this problem by using the fact that bacterial mRNA has an extremely short half-life, averaging only a few minutes. We reasoned that by targeting bacterial mRNA by a reverse transcriptase PCR (RT-PCR), a positive signal would indicate the presence of a recently viable organism. To test our hypothesis, we chose to target the mRNA coding for the ubiquitous 85B antigen of mycobacteria. After partially sequencing the gene coding for 85B, we developed primers that were specific for *Mycobacterium tuberculosis*. In a single-tube, nested, RT-PCR (STN RT-PCR), these primers detected fewer than 40 CFU in spiked sputum samples and as few as 12 CFU in clinical sputum specimens. The sensitivity of STN RT-PCR with smear-negative samples was as good as that of culture. The specificity was 100%. More importantly, when *M. tuberculosis* was cultured with and without 1 μg of isoniazid per ml, this assay could distinguish between those cultures which contained the antibiotic and those which did not. Subcultures on Lowenstein-Jensen agar confirmed the viability assessments of the STN RT-PCR. Control experiments demonstrated that isoniazid did not inhibit the RT-PCR. In addition, when an IS6110-targeted, DNA PCR was used to examine the same samples, all samples through 13 days (the last sample) continued to be positive, irrespective of whether isoniazid was present, thereby demonstrating the superiority of an mRNA target in the detection of mycobacterial viability.—Authors' Abstract

**Kolattukudy, P. E., Fernandes, N. D. Azad, A. K., Fitzmaurice, A. M. and Sirakova, T. D.** Biochemistry and molecular genetics of cell-wall lipid biosynthesis in mycobacteria. Mol. Microbiol. **24** (1997) 263–270.

Tuberculosis and other mycobacterial infections are the most serious infectious diseases in terms of human fatalities. The high content of unique cell-wall lipids helps these organisms to resist antimicrobial drugs and host defenses. The biosynthesis of these lipids is discussed briefly. The recent advances in recombinant DNA technology have begun to help to elucidate the nature of some of the enzymes involved in this process and the genes that encode them. Gene disruption and other molecular genetic technologies are beginning to provide new approaches to test for the biological functions of these gene products and may lead to identification of new antimycobacterial drug targets.—Authors' Summary

**Ma, Y., Mills, J. A., Belisle, J. T., Vissa, V., Howell, M., Bowlin, K., Scherman, M. S. and McNeil, M.** Determination of the pathway for rhamnose biosynthesis in mycobacteria: cloning, sequencing and expression of the *Mycobacterium tuberculosis* gene encoding α-D-glucose-1-phosphate thymidylytransferase. Microbiology **143** (1997) 937–945.

The mycobacterial cell-wall core consists of an outer lipid layer of mycolic acids connected, via arabinogalactan polysaccharide, to an inner peptidoglycan layer. An α-L-rhamnopyranosyl residue has been shown to be a key component linking the mycolated arabinogalactan to the peptidoglycan and, therefore, the biosynthesis of L-rhamnose (Rha) in mycobacteria was investigated as the first step of developing inhibitors of its biosynthesis. Biochemical assays were used to show that dTDP-Rha was synthesized in *Mycobacterium smegmatis* from α-D-glucose 1-phosphate (α-D-Glc-1-P) and dTTP by the same four enzymic steps used by *Escherichia coli* and other bacteria. PCR primers based on consensus regions of known sequences of the first enzyme in this series, α-D-Glc-1-P thymidylyltransferase (RfbA) were used to amplify *rfbA* DNA from *M. tuberculosis*. The entire *rfbA* gene was then cloned and sequenced. The deduced amino-acid sequence revealed

a 31,362 Da putative protein product which showed similarity to RfbA proteins of other bacteria (59% identity to that found in *E. coli*). Sequencing of DNA flanking the *rfbA* gene did not reveal any of the other *rfb* genes required for dTDP-Rha biosynthesis. Therefore, the four Rha biosynthetic genes are not clustered in *M. tuberculosis*. The enzymic activity of the sequenced gene product was confirmed by transformation of *E. coli* with pBluescript KS(–) containing the *rfbA* gene from *M. tuberculosis*. Analysis of enzyme extracts prepared from this transformant revealed an 11-fold increase in α-D-Glc-1-P thymidylyltransferase activity.—Authors' Abstract

**Marcinkeviciene, J. and Blanchard, J. S.** Catalytic properties of lipoamide dehydrogenase from *Mycobacterium smegmatis*. Arch. Biochem. Biophys. **340** (1997) 168–176.

Lipoamide dehydrogenase from *Mycobacterium smegmatis* was purified to homogeneity over 60-fold. Of 20 amino acid residues identified at the amino terminus of the enzyme, 18 and 17 were identical to the sequences of *M. leprae* and *Pseudomonas fluorescens* lipoamide dehydrogenases, respectively. The visible spectrum of the isolated enzyme was characteristic of a flavin in apolar environment. Reduction of the enzyme with dithionite results in the appearance of an absorbance shoulder at 530–550 nm, suggesting that reducing equivalents of the two-electron reduced enzyme reside predominantly on the redox-active disulfidedithiol. The kinetic mechanism of the forward ($NAD^+$ reducing) and reverse (NADH oxidizing) reactions proved difficult to study due to severe substrate inhibition by $NAD^+$ and NADH. The rate of lipoamide reduction was found to depend upon the $NAD^+$/NADH ratio, with the reaction being activated at low ratios and inhibited at high ratios. The use of 3-acetylpyridine adenine dinucleotide allowed initial velocity kinetics to be performed and revealed that the kinetic mechanism is ping pong. In addition to catalyzing the reversible oxidation of dihydrolipoamide, the enzyme displayed high oxidase activity (30% of the lipoamide reduction rate), hydrogen and t-butyl peroxide reductase activity (10% of the lipoamide reduction rate), and both naphthoquinone and benzoquinone reduction (similar to 200% of the lipoamide reduction rate). The enzyme failed to catalyze the redox cycling of nitrocompounds, but could anaerobically reduce nitrofurazone. The lipoamide-reducing reaction was reversibly inactivated by sodium arsenite, but no decrease in diaphorase activity was observed under these conditions.—Authors' Abstract

**Movahedzadeh, F., Colston, M. J. and Davis, E. O.** Characterization of *Mycobacterium tuberculosis* LexA: recognition of a Cheo (*Bacillus*-type SOS) box. Microbiology **143** (1997) 929–936.

The gene coding for the *Mycobacterium tuberculosis* homolog of LexA has been cloned and sequenced. Amino acids required for autocatalytic cleavage are conserved, whereas those important for specific DNA binding are not, when compared with *Escherichia coli* LexA. The transcriptional start site was mapped and a DNA sequence motif was identified which resembled the consensus Cheo box sequence involved in the regulation of DNA-damage-inducible genes in *Bacillus subtilis*. The *M. tuberculosis* LexA protein was overexpressed in *E. coli* and purified by means of a His tag. The purified LexA was shown to bind to the Cheo box sequence found upstream of its own gene.—Authors' Abstract

**Nakamura, M.** [Effects of glycerin and dextran on maintenance of the activity of *Mycobacterium leprae* in a cell-free liquid medium.] Jpn. J. Lepr. **65** (1996) 94–99. (in Japanese)

The author has previously indicated that the activity (ATP values extracted from collected cells) of *Mycobacterium leprae* could be maintained in phosphate buffer (pH 7.0) containing fetal calf serum (10%) for more than 4 weeks incubated at 30°C. The present paper describes how the activity of cells of *M. leprae* is prolonged and somewhat stimulated when glycerin and dextran are added to the buffer serum system. The optimal concentrations for glycerin and dextran are

2% and 1%, respectively. In addition, it was found that dextran of 200–300 kDa is much more effective than that of 100–200 kDa.—Trop. Dis. Bull. **94** (1997) 128

**Pavelka, M. S., Weisbrod, T. R. and Jacobs, W. R.** Cloning of the dapB gene, encoding dihydrodipicolinate reductase, from *Mycobacterium tuberculosis.* J. Bacteriol. **179** (1997) 2777–2782.

Diaminopimelate (DAP) is used by bacteria for the synthesis of lysine. In many species of bacteria, including mycobacteria, DAP is also used for peptidoglycan biosynthesis. In this report we describe the cloning of the dapB gene encoding dihydrodipicolinate reductase (DHPR), which catalyzes a key branch point reaction in the bacterial DAP biosynthetic pathway, from *Mycobacterium tuberculosis.* Analyses of the DapB proteins from different bacterial species suggest that two different classes of DHPR enzymes may exist in bacteria.—Authors' Abstract

**Purwantini, E., Gillis, T. P. and Daniels, L.** Presence of F-420-dependent glucose-6-phosphate dehydrogenase in *Mycobacterium* and *Nocardia* species, but absence from *Streptomyces* and *Corynebacterium* species and methanogenic *Archaea.* FEMS Microbiol. Lett. **146** (1997) 129–134.

A range of organisms known to contain F-420 or to be relatives of mycobacteria were examined for F-420-dependent glucose-6-phosphate dehydrogenase (FGD) and NADP-dependent glucose-6-phosphate dehydrogenase (NADP-G6PD) activities. All free-growing *Mycobacterium* species examined (including a virulent *Mycobacterium tuberculosis* strain) had FGD activities of 0.014–0.418 $\mu$mol min$^{-1}$ mg protein$^{-1}$, and NADP-G6PD activities of 0.013– 0.636 $\mu$mol min$^{-1}$ mg$^{-1}$. Armadillo-grown *M. leprae* had FGD activity of 0.008 $\mu$mol min$^{-1}$ mg$^{-1}$, but no detectable NADP-G6PD activity. *Nocardia* species also had FGD activity (0.088–0.154 $\mu$mol min$^{-1}$ mg$^{-1}$). *Streptomyces* and *Corynebacterium* species had no FGD, but had NADP-G6PD. Methanogenic *Archaea* had neither activity.—Authors' Abstract

**Santos, A. R., Nery, J. C., Duppre, N. C., Gallo, M. E. N., Filho, J. T. G., Suffys, P. N. and Degrave, W. M.** Use of the polymerase chain reaction in the diagnosis of leprosy. J. Med. Microbiol. **46** (1997) 170–172.

One of the main limitations for successful epidemiological control of leprosy is the lack of a method for its diagnosis in subclinical cases. Because of the long incubation period of the disease, liberation and spread of *Mycobacterium leprae* during subclinical stages—principally on cases of untreated multibacillary forms of leprosy—constitute the main source of infection. This report describes the use of the polymerase chain reaction (PCR) for the detection of *M. leprae* in different types of tissue samples (blood, lymph, nasal secretion and hair) from an individual who was suspected of having leprosy. Although no conclusive diagnosis could be made by traditional diagnostic methods, the individual was found to be infected with *M. leprae* after amplification of the bacterial DNA.—Authors' Abstract

**Scorpio, A., Lindholm Levy, P., Heifets, L., Gilman, R., Siddiqi, S., Cynamon, M. and Zhang, Y.** Characterization of pncA mutations in pyrazinamide-resistant *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **41** (1997) 540–543.

Pyrazinamide (PZA) is a first-line drug for short-course tuberculosis therapy. Resistance to PZA is usually accompanied by loss of pyrazinamidase (PZase) activity in *Mycobacterium tuberculosis.* PZase converts PZA to bactericidal pyrazinoic acid, and the loss of PZase activity is associated with PZA resistance. The gene (pncA) encoding the *M. tuberculosis* PZase has recently been sequenced, and mutations in pncA were previously found in a small number of PZA-resistant *M. tuberculosis* strains. To further understand the genetic basis of PZA resistance and determine the frequency of PZA-resistant strains having pncA mutations, we analyzed a panel of PZA-resistant clinical isolates and mutants made *in vitro.* Thirty-three of 38 PZA-resistant clinical isolates had pncA mutations. Among the five strains that did not contain

pncA mutations, four were found to be falsely resistant and one was found to be borderline resistant to PZA. The 33 PZA-resistant clinical isolates and 8 mutants made *in vitro* contained various mutations, including nucleotide substitutions, insertions, or deletions in the pncA gene. The identified mutations were dispersed along the pncA gene, but some degree of clustering of mutations was found at the following regions: Gly132–Thr142, Pro69–Leu85, and Ile5–Asp12. PCR-single-strand conformation polymorphism (SSCP) analysis was shown to be useful for the rapid detection of pncA mutations in the PZA-resistant strains. We conclude that a mutation in the pncA gene is a major mechanism of PZA resistance and that direct sequencing by PCR or SSCP analysis should help to rapidly identify PZA-resistant *M. tuberculosis* strains.—Authors' Abstract

**Shetty, V. P., Uplekar, M. W. and Antia, N. H.** Primary resistance to single and multiple drugs in leprosy—a mouse footpad study. Lepr. Rev. **67** (1996) 280–286.

Skin biopsy homogenates obtained from three cases of lepromatous leprosy with no prior history of antileprosy treatment were tested in the mouse foot pad for the sensitivity of *Mycobacterium leprae* to multiple drugs. One of the inocula was sensitive to all three drugs tested using the highest concentration each of DDS 0.01 g%, RFP 0.03 g% and CLF 0.01 g%. The second inocula showed growth in the presence of 0.01 g% DDS only. While the third inocula (Pt. KU) tested resistant to all three drugs in the first, i.e., man to mouse, as well as in the second passage, i.e., mouse to mouse.—Authors' Summary

**Sreevatsan, S., Pan, X., Zhang, Y., Kreiswirth, B. N. and Musser, J. M.** Mutations associated with pyrazinamide resistance in pncA of *Mycobacterium tuberculosis* complex organisms. Antimicrob. Agents Chemother. **41** (1997) 636–640.

A gene (pncA) with mutations associated with pyrazinamide resistance in *Mycobacterium tuberculosis* complex members was characterized in 67 pyrazinamide-resistant and 51 pyrazinamide-susceptible isolates recovered from diverse geographic localities and anatomic sites and typed by IS6110 profiling. All pyrazinamide-susceptible organisms had identical pncA alleles. In striking contrast, 72% of the 67 resistant organisms had pncA mutations that altered the primary amino acid sequence of pyrazinamidase. A total of 17 previously undescribed mutations were found, including upstream mutations, missense changes, nucleotide insertions and deletions, and termination mutations. The mutations were arrayed along virtually the entire length of the gene. These data are further evidence that most drug resistance in *M. tuberculosis* is due to simple mutations occurring in chromosomally encoded genes rather than to acquisition of resistance genes by horizontal transfer events.—Authors' Abstract

## Experimental Infections

**Banerjee, D. K., McDermott-Lancaster, R. D. and McKenzie, S.** Experimental evaluation of possible new short-term drug regimens for treatment of multibacillary leprosy. Antimicrob. Agents Chemother. **41** (1997) 326–330.

Groups of nude mice, with both hind foot pads infected with $10^8$ *Mycobacterium leprae* organisms, were treated with 4-week courses of different drug combinations. The effect of each treatment on each group was evaluated by subinoculating foot pad homogenates from the treated mice into groups of normal and nude mice for subsequent regrowth, assessed 1 year later. A combination of rifampin (RMP) with clarithromycin (CLARI), minocycline (MINO), and ofloxacin (OFLO) resulted in the complete killing of *M. leprae* after 3 weeks of treatment. A combination of sparfloxacin (SPAR) and RMP also resulted in a similar bactericidal effect after 3 weeks of treatment. Other drug combinations showed variable effects.

Very little or no effect was observed with any regimen if the treatment was given for less than 2 weeks. World Health Organization multidrug therapy (WHO/MDT) given for 8 weeks was as effective as the two combinations described above. The results suggest that multidrug combinations consisting of RMP-OFLO (or SPAR)-CLARI (and/or MINO) are as effective as the WHO/MDT for the treatment of experimental leprosy. Moreover, they imply that these combinations, which were found to be active in a 4-week experimental treatment protocol, could be administered as treatment to patients for a period of time shorter than the present 2-year regimen without a loss of effectiveness.—Authors' Abstract

**Singh, N., Birdi, T. J. and Antia, N. H.** Nerve growth factor production and expression of p75 by Schwann cells and neurofibroblasts in response to *M. leprae* infection and macrophage secretory products. Neuropathol. Appl. Neurobiol. **23** (1997) 59–67.

This study describes the changes occurring *in vitro* in nerve growth factor (NGF) production and expression of p75 by murine Schwann cells and neurofibroblasts, following infection with *Mycobacterium leprae* and in the presence of macrophage secretory products, using a semiquantitative ELISA. These parameters are compared in two strains of mice, Swiss white (SW) and C57BL/6, as they differ in their response to *M. leprae* infection: C57BL/6 is the "resistant" strain. On infection, NGF levels remained unaltered in Schwann cells from both strains, while fibroblasts from C57BL/6 strain showed an increase in NGF production. Expression of p75 by Schwann cells was decreased on infection in both strains of mice. *In vivo,* this opposing effect of infection on NGF production and p75 expression by Schwann cells and neurofibroblasts may result in suboptimal amounts of NGF reaching neurons of the affected leprous nerves. Macrophage secretory products suppressed the production of NGF by infected neurofibroblasts from SW strain mice and the expression of p75 in Schwann cells from both strains. These results indicate that macrophages do not assist in nerve repair in leprosy and the differences in response to macrophage secretory products in the two strains suggest that different mechanisms of nerve repair operate in SW and C57BL/6 mice and presumably in lepromatous and tuberculoid patients.—Authors' Abstract

## Epidemiology and Prevention

**Abou Zeid, C., Gares, M. P., Inwald, J., Janssen, R., Zhang, Y., Young, D. B., Hetzel, C., Lamb, J. R., Baldwin, S. L., Orme, I. M., Yeremeev, V., Nikonenko, B. V. and Apt, A. S.** Induction of a type 1 immune response to a recombinant antigen from *Mycobacterium tuberculosis* expressed in *Mycobacterium vaccae*. Infect. Immun. **65** (1997) 1856–1862.

A 19-kDa lipoprotein from *Mycobacterium tuberculosis* was expressed as a recombinant antigen in the nonpathogenic mycobacterial host strain *M. vaccae*. Immunization of mice with the recombinant *M. vaccae* resulted in induction of a strong type 1 immune response to the 19-kDa antigen, characterized by immunoglobulin G2a (IgG2a) antibodies and gamma interferon (IFN-$\gamma$) production by splenocytes. Immunization with the same antigen in incomplete Freund's adjuvant induced a strong IgG1 response with only low levels of IFN-$\gamma$. Subsequent intravenous and aerosol challenges of immunized mice with virulent *M. tuberculosis* demonstrated no evidence of protection associated with the response to the 19-kDa antigen: in fact, the presence of the recombinant 19-kDa antigen abrogated the limited protection conferred by *M. vaccae* (vector control). The recombinant *M. vaccae* system is a convenient approach to induction of type 1 responses to *M. tuberculosis* antigens. However, the unexpected reduction in protective efficacy of *M. vaccae* expressing the 19-kDa antigen high-

lights the complexity of testing recombinant subunit vaccines and the need for a better understanding of the immune mechanisms required for effective vaccination against tuberculosis.—Authors' Abstract

**Castellazzi, Z., Batista, P. and Bogaert, H.** [Results of an operation to detect leprosy cases by a massive examination of the population done in the city of Pedernales, June 1996.] Rev. Dominicana Dermatol. **23** (1996) 27–30. (in Spanish)

Report of the results of an operation to detect cases of leprosy made by the South East Unit in the city of Pedernales, Dominican Republic, June 1996. This was executed by six doctors, ten public health assistants, two inspectors and the general supervisor of inspectors in a period of 2 days. The total figure examined reached 4079, finding 16 persons suspected with the ailment, 8 of which resulted with leprosy.—Authors' English Summary

**Duangajna, P.** Leprosy control program in Thailand. Jpn. J. Lepr. **65** (1996) 88–93.

The author discusses the Leprosy Control Programme in Thailand with consideration of the following aspects: initial isolation phase (1909–1955); vertical control program (1956–1970); integration approach (1970–1981); primary health care approach (1984–present); multidrug therapy implementation; elimination of leprosy problem in Thailand; elimination activities; elimination goal; action following success in elimination programme.—Trop. Dis. Bull. **94** (1997) 129

**Joko, S., Numaga, J., Masuda, K., Namisato, M. and Maeda, H.** [HLA class II alleles and leprosy (Hansen's disease) classified by WHO-MDT criteria.] Jpn. J. Lepr. **65** (1996) 121–127. (in Japanese)

Human leukocyte antigens (HLA) class II alleles were analyzed in Japanese leprosy patients to ascertain whether immunogenetic differences exist among the forms of leprosy in classification of World Health Organization-recommended multidrug therapy (WHO/MDT). The subjects were 86 unrelated Japanese leprosy patients, including 62 multibacillary leprosy (MBL), 24 paucibacillary leprosy (PBL). Controls were 114 unrelated healthy subjects. Genotyping of HLA class II alleles was performed by using the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and PCR-restriction fragment length polymorphism (RFLP) methods. The frequencies of HLA-DRB1*1501, *1502 and DRB5*0101, *0102 and DQA1*0102 and DQB1*0602 were significantly increased in all the leprosy patients combined (44.2%, 34.9%, 44.2%, 34.9%, 53.4% and 41.9%, respectively) as compared with the control subjects (14.0%, 21.1%, 14.0%, 21.1%, 27.2% and 13.2%, respectively). On the other hand, the frequencies of HLA-DRB1*0405, *0803, *0901 and DQA1*03 and DQB1*0401 were significantly decreased in the combined patients (10.5%, 5.8%, 16.3%, 41.9% and 9.3%, respectively) as compared with the control subjects (29.8%, 17.5%, 30.7%, 78.1% and 29.8%, respectively). When MBL and PBL patients were compared, the frequencies of HLA-DRB1*1501, DRB5*0101 and DQB1*0602 were significantly increased in the MBL patients (51.6%, 51.6% and 48.4%, respectively) as compared with the PBL patients (25.0%). The results suggest that HLA-DRB1*1501, DRB5*0101 and DQB1*0602 contribute to the susceptibility to Japanese MBL.—Trop. Dis. Bull. **94** (1997) 128–129

**Li, J., *et al.*** [Analysis of 3642 newly detected cases of leprosy in Guizhou Province.] China Lepr. J. **12** (1996) 240–241. (in Chinese)

In the period of 1986 to 1993, 3642 new cases of leprosy have been found, mainly with clue survey (34.4%), outpatient service and self-report by patient, in Guizhou Province. Finding rate decreased from 2.21/100,000 in 1986 to 0.84/100,000 in 1993. The value of MB/PB and proportion of children in new patients did not change significantly. Ratio of male to female in the patients declined from 4.8 to 3.1. Disability rate of degree II plus III was about 20%.

The authors concluded that leprosy is still endemic in this province, so control work for it has to be strengthened there.—Authors' English Abstract

**Rajalingam, R., Singal, D. P. and Mehra, N. K.** Transporter associated with antigen-processing (TAP) genes and susceptibility to tuberculoid leprosy and pulmonary tuberculosis. Tissue Antigens **49** (1997) 168–172.

We have studied TAP polymorphism in a panel of 40 healthy individuals, 57 patients with pulmonary tuberculosis (PTB) and 50 with tuberculoid (TT) leprosy from North India. Only TAP2-A/F occurred with a significantly increased frequency in PTB patients as compared to controls (82.5% vs. 52.5%, $p < 0.002$, $p < 0.01$) giving a high relative risk of 4.3. On the other hand, TAP2-B was significantly increased in TT leprosy as compared to controls (76% vs. 47.5%, $p < 0.003$, RR = 3.5) particularly in patients positive for HLA-DR15 than controls carrying DR15 (77.5% vs. 50%, $p < 0.03$, RR = 3.4). Further, TAP2-B allele was positively associated with DR15 negative PTB patients as compared to the DR15 positive group (43.8% vs. 17.1%, $p < 0.04$, RR = 0.3). This study along with our earlier studies on HLA association in mycobacterial diseases suggests that in addition to HLA-DR15, alleles in the TAP2 region influence susceptibility to PTB and TT leprosy.—Authors' Abstract

**Sun, X., *et al.*** [Analysis of new cases of leprosy detected for 1985 to 1993.] China Lepr. J. **12** (1996) 227–228. (in Chinese)

In Weifang City and Linyi Prefecture, Shandong Province, China, among newly detected leprosy patients in the last years, the age at onset of the disease increased, infectious source was unknown in most of them, disease duration shortened and disability was alleviated.—Authors' English Abstract

**Sun, X., *et al.*** [Factors influencing on the disease duration in leprosy.] China Lepr. J. **12** (1996) 229–234. (in Chinese)

Single factor and multivariate analyses of disease duration in 239 leprosy patients with a mean disease duration of 32.3 months showed that six factors had exerted main effect on it, including times of seeing doctor before suspected the disease, psychological reaction of the patient and their family to having the disease, way and time they were found and their sex. The authors put forward measures to intervene in these factors for early case-finding.—Authors' English Abstract

**Sun, X., *et al.*** [On case-finding ways and their effects in leprosy.] China Lepr. J. **12** (1996) 225–227. (in Chinese)

There are two methods of case-finding in leprosy, i.e., active and passive ones. For the last years the patients detected by the passive method have been increasing, but the active one has very low cost-effectiveness and most of the patients found with it had severe disability. The authors suggest that health eduction should be strengthened for promoting early case-finding.—Authors' English Abstract

**Tiendrebeogo, A., Guedonon, A., Zerbo, P. J., Djakeaux, S., Berthe, A., Benani, Y. C., Souley, K., Sylla, P. M. and Napo, T.** [Leprosy and its control in 1994 in the eight member countries of the OCCGE.] Med. Afr. Noire **43** (1996) 107–111. (in French)

This article provides data for the leprosy situation in 1994 in the eight West African countries of the OCCGE (Organisation pour la Coordination et la Coopération pour la lutte contre les Grandes Endémies): Benin, Burkina Faso, Côte d'Ivoire, Mali, Mauritania, Niger, Senegal, and Togo (figures are for 1993 for the last country). Overall, the case detection rate (per 10,000) was 1.33, ranging from 0.49 in Senegal to 2.11 in Mali; the prevalence rate (per 10,000) was 5.69, ranging from 1.93 in Burkina Faso to 14.71 in Mali. Although leprosy remained a public health problem in the region, 69% of registered cases were being treated with multidrug therapy (range, 48% in Niger to 100% in Benin, Burkina Faso, Côte d'Ivoire, Senegal, and Togo.)—Trop. Dis. Bull. **93** (1996) 1034

**World Health Organization.** Progress towards the elimination of leprosy as a public health problem. Wkly. Epidemiol. Rec. **71** (1996) 149–156.

The prevalence of leprosy worldwide was reduced by 28% between 1995 and 1996, compared with 27% between 1994 and 1995. These reductions can be explained by the conjunction of the following factors: wider implementation of MDT, fixed duration of treatment, and updating of the leprosy registers. The following topics are reviewed: estimated and registered prevalence; detection of leprosy cases; achievements with MDT; progress toward the elimination of leprosy (situation in the top 16 endemic countries); and the leprosy situation in the most endemic countries.—Trop. Dis. Bull. **94** (1997) 128

**Yu, A., *et al.*** [Evaluation of leprosy control in Liaoning Province.] China Lepr. J. **12** (1996) 243–245. (in Chinese)

Since 1949 to 1994, 1952 cases of leprosy have been registered in Liaoning Province, China, distributed in 93 counties and cities and the most (55%) were in Dalian and Yingkou cities of the southern region. 1914 cases have been cured. In 1994 there were only 28 active cases under MDT, the prevalence was below 0.009‰ and the incidence below 0.045/100,000. In the last 5 years 10 new patients have been found (MB 7 and PB 3); those with degree 1 and 2 disability account for 50%. In 1994 MOPH approved that the goal of basically eradicating leprosy has been reached in the Province. The leprosy control work will be continued until all eradication of leprosy.—Authors' English Abstract

**Zhou, L., *et al.*** [Preliminary evaluation of a SAPEL in a town, Yunnan.] China Lepr. J. **12** (1996) 248–250. (in Chinese)

A SAPEL, according to Hanoi Declaration, was carried out by a medical team under the guidance of Dr. H. Y. Li in a minority nationality town with a population of 45,000, Yunnan, and has detected nine new leprosy patients and given them MDT after training medical workers and giving wide health education in the town and villages for 6 days. The detection rate is 0.2‰ and there were 11 registered cases, so the prevalence rate is 0.44‰ but not 0.24‰ as known formerly.—Authors' English Abstract

## Rehabilitation

**Bari, M. M., Islam, A. K. M. S. and Haque, A. K. M. A.** Surgical reconstruction of leprotic foot-drop. Lepr. Rev. **67** (1996) 200–202.

At the Leprosy Control Institute and Hospital in Dhaka, Bangladesh, the authors operated on 25 patients for correction of foot drop due to leprosy between March 1992 and July 1994. The method used was circumtibial transfer of the tibialis posterior to the tendons of extensor hallucis longus and the extensor digitorum longus in the foot together with lengthening of the Achilles tendon. The results were satisfactory in 20 of these cases as judged by adequate restoration of heel-toe gait and of active dorsiflexion. The follow-up period ranged from 6 months to 2 years. Inadequate postoperative physiotherapy was the reason for unsatisfactory results in 5 cases.—Trop. Dis. Bull. **93** (1996) 1036

**Beine, A.** Prevention of post-operative "sublimis minus" deformity by modified surgical procedure at the donor finger. Indian J. Lepr. **69** (1997) 33–41.

"Sublimes minus" deformity occurs quite often as a postoperative deformity in the donor finger after transfer of the flexor superficialis tendon, e.g., for paralytic claw finger correction. Our experience with a new procedure to avoid this outcome is described here. Long-term results in 35 cases are very encouraging and the new procedure promises to be useful to prevent sublimis minus deformity It also opens up a wider range of discretion given to hand sur-

geons to use the sublimis tendon more freely for transfers.—Author's Abstract

**Cakiner, T , Yuksel, A., Senal Egit, A., Cagri, G., Karacorlu, M. and Kultur, A.** The extent of leprosy-related disabilities in Istanbul Leprosy Hospital, Turkey. Lepr. Rev. **68** (1997) 43–49.

This study was carried out between January and December 1992 at the Istanbul Leprosy Hospital. Seven-hundred-eleven leprosy patients were evaluated according to their age, gender and type of disease and disability according to the WHO disability grading system (1980). There were 527 males (74.2%) and 184 females (25.8%) in the group. The average age was 50.0 ± 13.5 years and the average duration of disease was 25.9 ± 13.2 years. Six-hundred-seventy-eight patients (95.4%) were suffering from borderline (BL) and lepromatous (LL) leprosy.

The extent of disabilities was very high in 711 leprosy patients. It was found that 539 of the patients (75.8%) had eye disabilities, 511 of them (71.8%) had hand disabilities, 521 of them (73.3%) had foot disabilities.

The most frequent eye, hand and foot disabilities were a decrease of vision (52.7), acute or chronic iridocyclitis (48.8%), slightly marked corneal sensory loss (43.2%), mobile claw hand (33.3%), palmar insensitivity (16.3%), plantar ulcer (37.2%) and plantar insensitivity (19.8%).

Eye deformities were the most common of the three affected areas in this study.—Authors' Summary

**Castellazzi, Z.** [Diagnosis and treatment of physical disabilities in leprosy.] Rev. Dominicana Dermatol. **21** (1994) 9–17. (in Spanish)

The author defines the concept of rehabilitation in leprosy, emphasizing the educative part which is considered basic. All the personnel that work with leprosy must know how to give it in order to avoid most of the grade 2 disabilities. It is also listed all the causes of physical disabilities in the leprosy patient, showing the neurological involvement and all the manifestations in face, hands and feet. A classification of all disabilities was made and they are divided into primaries and secondaries, with their morphological and functional expression, and there is also a table with all the elements that allow the suspicious and accurate diagnosis from the initial lesion on each pheripheral nerve trunk. It is also reported the general concepts about the management of the physical disabilities, ending with a synthesis of each treatment.—Author's English Abstract

**Castellazzi, Z., Bogaert, H., Isa, R., Ledesma, R. and Reyes, A. V.** [Childhood leprosy: incidence of cases less than 15, 1990–1994; a clinico-epidemiologic study.] Rev. Dominicana Dermatol. **22** (1995) 7–12. (in Spanish)

The authors did an epidemiological clinical study of 266 cases of leprosy which occurred in minors aged 15 who were diagnosed in the Instituto Dermatológicao y Cirugía de Piel during the 5-year period 1990–1994. Analysis is made on the incidence by 100,000 inhabitants, percentage over the total persons affected diagnosed each year, clinical forms and age groups, disabilities grade II, sources of infection, time elapses between the apparition of the first ailment and diagnosis.—Authors' English Summary

**Ebenezer, M. and Sundar Rao, P. S. S.** Alternative approaches for the prevention of disability in leprosy. Lepr. Rev. **68** (1997) 50–54.

Cost-effective programs for the prevention of disabilities in leprosy require active involvement of the patients and their families as well as an integrated team approach. This paper presents the views and recommendations of a group of 35 experienced leprologists who met at a workshop, reviewed the current scenario and worked out specific objectives, strategies and the reorganization required in the existing infrastructure. Three tiers of workers are suggested: village volunteer; paramedical worker; and the professionals at the base hospital. All three levels should work together at the start of a program well as for

periodic monitoring and evaluation.—Authors' Summary

**Jiang, Z., *et al.*** [Comprehensive control of complex plantar ulcers in leprosy.] China Lepr. J. **12** (1996) 255–256. (in Chinese)

Through teaching patients self-care for their feet with complex plantar ulcers and making them persist in doing so, out of 70 ulcers in 52 cases 58 ulcers of 44 cases have healed up for 2 to 5 months, but 10 ulcers relapsed after healing because of overwalking. The authors suggest that leprosy control services should have a group of staff, including a surgeon, nurse, shoe-making worker and sociologist, for the prevention and treatment of plantar ulcers.—Authors' English Abstract

**Kazen, R. O.** A double synergistic approach to correction of the intrinsic minus hand. Indian J. Lepr. **69** (1997) 53–61.

In the search of a method for the correction of the intrinsic minus hand with simplified postoperative physiotherapy training, preferably dynamic methods acting in a synergistic way, two methods were identified, one correcting clawing in extension, the other correcting clawing in flexion under strain. Forty-three patients (53 hands) were operated upon using a combination of the methods. The immediate postoperative results showed that 50 of the hands had satisfactory result. Twenty patients (26 hands) were traced and reviewed after 0.5–5.5 years (average 2.75 years) and 14 of these hands showed good, 11 fair, and 1 poor results. All patients reported that their hand function had improved after correction. Postoperatively, the physiotherapy staff were only required to exercise active extension and flexion of the fingers. The advantages and disadvantages of the approach are discussed. The method requires an experienced surgeon but the postoperative training is simple and there is no need for re-education of transferred tendons. I have found the method useful for patients with difficulties in re-education and for patients in programs where the physiotherapy back up is less developed.—Author's Abstract

**Patond, K. R., Betal, B. D. and Kumar, A.** Surgical correction of claw fingers in leprosy using flexor superficialis direct lasso procedure. Indian J. Lepr. **69** (1997) 25–32.

One tendon of flexor digitorum superficialis split into two or four tails and each tail looped around the AI pulley of one affected finger was used for correcting intrinsic minus deformity of fingers in 144 patients. This operation has the advantage of retaining superficialis tendons of the other fingers for better power grip and avoiding swan-neck deformity. Satisfactory results were noticed in 81 of the 99 patients (82%) followed up. The remaining 18 patients showed a variety of problems, such as swan neck/check rein deformity in the donor finger, skin contracture and recurrence of clawing.—Authors' Abstract

**Rath, S.** Flexor aponeurotic release for resistant adaptive shortening of long flexors in claw hands in leprosy. Indian J. Lepr. **69** (1997) 101–107.

Adaptive shortening of long flexors (ASLF) is a consequence of long-standing neglected claw finger deformity. While adaptive shortening of muscle fibers is correctable by physiotherapy, concurrent shortening of fascia/aponeurosis/intermuscular septum, composed of inelastic collagen fibers is not. Surgical excision of these structures has been advised in ASLF in cerebral palsy. This procedure in which a 3–4-cm-wide band of deep fascia of the forearm, about 6-cm distal to the medial epicondyle, along with the intermuscular septum is excized was tried in seven patients having severe or moderately severe ASLF with good results. This procedure is worth a more extensive trial.—Author's Abstract

**Soares, D. and Chew, M.** Temporalis muscle transfer in the correction of lagophthalmos due to leprosy. Lepr. Rev. **68** (1997) 38–42.

In the correction of lagophthalmos due to leprosy, neuritis temporalis muscle transfer (TMT) is used to provide a motor to assist

in lid closure. This study of TMT in 51 eyes was carried out to assess the effectiveness of TMT in achieving lid closure and corneal protection. The average lid gap preoperatively on light closure was 7.3 mm which was reduced to 3.2 mm on final follow up. The average lid gap pre-operatively on tight closure was 5.3 mm which was reduced to 0.4 mm at final follow up. It is possible to train patients with partial or total anesthesia of the cornea in a visual Think-Blink reflex. The common complications encountered were ectropion in 6 eyes (12%) and ptosis in 3 eyes (6%).—Authors' Summary

**Teo, T. C. and Richard, B. M.** The distally based posterior interosseous fasciocutaneous island flap in reconstruction of the hand in leprosy. Indian J. Lepr. **69** (1997) 93–100.

In leprosy a functionally useless hand can be the sequelae of resorption of the sensation-impaired thumb and fingers resulting from repeated trauma and infection. When this process shortens the thumb to the level of the proximal phalanx or metacarpal, the effect is to produce a relatively shallow first web space which, together with the shortening, can prevent the most basic hand maneuver of a pincer or pinch grip. The reconstructive procedure commonly used in this situation is to widen and deepen the web space with a z-plasty combined with excision of the second metacarpal but the result can be inadequate. We have used the posterior interosseous fasciocutaneous island flap, both as a simple and a compound flap, to solve this challenging problem and we report here our experience with four patients.—Authors' Abstract

## Other Mycobacterial Diseases and Related Entities

**Alexander, L. N. and Wilcox, C. M.** A prospective trial of thalidomide for the treatment of HIV-associated idiopathic esophageal ulcers. AIDS Res. Hum. Retrovir. **13** (1997) 301–304.

Thalidomide appears to be highly effective for oropharyngeal aphthous ulcers in HIV-infected patients. However, there are limited data regarding the use of this drug for the treatment of HIV-associated idiopathic esophageal ulcer(s) (IEU). Twelve HIV-infected patients with esophageal symptoms and IEU as defined by previously proposed criteria were studied prospectively. Two of these patients had failed oral corticosteroid treatment, and two others had a previous history of IEU. Patients were treated with thalidomide (200 mg/day orally) for 28 days in an open label fashion. Clinical evaluation was performed weekly with endoscopic re-examination performed at the completion of treatment. After therapy, patients were followed clinically with endoscopy recommended for recurrent esophageal symptoms. Of the 12 treated patients, 11 (92%) had a complete symptomatic response; endoscopy in 11 patients at the completion of treatment showed 9 with complete ulcer healing, 1 partially healed, and 1 with no response. All responders were asymptomatic by day 28. The partial responder received an additional 1 month of thalidomide at 300 mg/day, resulting in complete endoscopic healing. The patient failing therapy received prednisone, but died prior to completing this therapy. On follow up to 20 months, six patients have died with no recurrence of IEU. Three patients had relapse of IEU, two of whom had a prior history of multiple recurrences of IEU; both of these patients relapsed within 2 months of completing thalidomide treatment. The drug was well tolerated without significant side effects. Thalidomide appears to be an effective and well-tolerated alternative to prednisone for the treatment of IEU.—Authors' Abstract

**Amaral, L., Kristiansen, J. E., Abebe, L. S. and Millett, W.** Inhibition of the respiration of multidrug resistant clinical isolates of *Mycobacterium tuberculosis* by thioridazine: potential use for initial ther-

apy of freshly diagnosed tuberculosis. J. Antimicrob. Chemother. **38** (1996) 1049–1053.

Chlorpromazine and thioridazine are phenothiazines employed in the treatment of psychosis. These agents inhibited the respiration of clinical isolates of *Mycobacterium tuberculosis* resistant to streptomycin, rifampin, isoniazid, ethambutol and/or pyrazinamid, all first line drugs. Since any adverse reaction to thioridazine is generally less severe than to chlorpromazine, the possibility is attractive that thioridazine may have a potential in the initial management of patients with newly diagnosed tuberculosis with an as yet undetermined antibiotic susceptibility profile.—Authors' Abstract

**Andersen, P.** Host responses and antigens involved in protective immunity to *Mycobacterium tuberculosis.* Scand. J. Immunol. **45** (1997) 115–131.

Tuberculosis (TB) is the largest single infectious cause of human mortality. The incidence of TB has remained high in most of the developing world and the disease has recently re-emerged as a public health problem in industrialized countries. The development of a new improved TB vaccine is a highly prioritized international research area, which has been further stimulated by the appearance of multidrug-resistant strains of *Mycobacterium tuberculosis.* The present status of the attempts to characterize the protective immune response to TB will be reviewed with special emphasis on recent progress in the identification and characterization of target molecules recognized by protective cells. This paper will focus on proteins released from live bacteria and discuss their role in the host-pathogen interaction and the ongoing attempts to use these molecules in TB subunit vaccines.—Author's Abstract

**Baranda, L., Torres-Alvarez, B., Cortes-Franco, R., Moncada, B., Portales-Perez, D. P. and Gonzales-Amaro, R.** Involvement of cell adhesion and activation molecules in the pathogenesis of erythema dyschromicum perstans (ashy dermatitis); the effect of clofazimine therapy. Arch. Dermatol. **133** (1997) 325–329.

Objectives: To assess the expression of several cell adhesion and lymphocyte activation molecules in erythema dyschromicum perstans lesions, and to evaluate the effect of clofazimine therapy on the expression of these molecules.

Design and Methods: A prospective study. Skin biopsy samples were obtained from patients before and after 3 months of clofazimine therapy, and the expression of cell adhesion and activation molecules was assessed by an immunohistochemical technique.

Setting: This study was performed in a clinical referral center and an immunology research laboratory.

Patients: We studied 6 patients with erythema dyschromicum perstans. A diagnosis was made on the basis of clinical and histological criteria. Two patients discontinued participation in the study: one because of adverse effects and the other for unknown reasons.

Interventions: Patients were treated with clofazimine, 100 mg/d, for 3 months.

Main Outcome Measures: Expression of cell adhesion and lymphocyte activation molecules in skin biopsy specimens before and after clofazimine therapy.

Results: Before clofazimine therapy, we detected a noticeable expression of intercellular adhesion molecule 1 and major histocompatibility complex class II molecules (HLA-DR) in the keratinocyte basal cell layer. In addition, CD36, a thrombospondin receptor that is not expressed by normal skin, was detected in the strata spinosum and granulosum. The dermal cell infiltrate expressed the activation molecule AIM/CD69 and the cytotoxic cell marker CD94. After clofazimine therapy, the expression of intercellular adhesion molecule 1 and HLA-DR disappeared, as well as the mononuclear cell infiltrate.

Conclusions: Our results suggest that some cell adhesion and activation molecules are involved in the pathogenesis of erythema dyschromicum perstans. Clofazimine appears to have an important effect on the inflammatory phenomenon of erythema dyschromicum perstans.—Authors' Abstract

**Baselga, E., Margall, N., Barnadas, M. A., Coll, P. and de Moragas, J.** Detection of *Mycobacterium tuberculosis* DNA in lobular granulomatous panniculitis (erythema induratum nodular vasculitis). Arch. Dermatol. **133** (1997) 457–462.

Objective: To determine, using polymerase chain reaction (PCR) amplification, if *Mycobacterium tuberculosis* complex DNA is present in the skin-biopsy specimens of lobular granulomatous panniculitis.

Design: A retrospective descriptive study.

Setting: A university-based hospital.

Patients: From the 65 patients included in the study, we examined 72 paraffin-embedded skin-biopsy specimens with a histologic diagnosis of erythema induratum or nodular vasculitis. The biopsy specimens were from the histopathological archives of the Departments of Dermatology and Pathology of the Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, from 1976 to 1994. Twenty-two biopsy specimens were excluded from the final analysis because we could not amplify the internal control.

Main Outcome Measures: Detection of a 123-base pair fragment of the IS6110 insertion sequence specific for *M. tuberculosis* complex.

Results: The results of PCR amplification were positive for *M. tuberculosis* complex DNA in 77% of the skin-biopsy specimens. No significant difference could be detected with respect to the age of the patients, ulceration of the nodules, reactivity to purified protein derivative, abnormal results of a chest X-ray examination, personal and family history of tuberculosis, and PCR results. The presence and degree of necrosis on histologic examination were significantly higher in the PCR-positive group ($p = 0.04$). None of the following variables were associated with PCR results: presence of vasculitis, degree of granulomatous infiltrates, number of giant cells, and presence of well-organized granulomas.

Conclusions: The DNA of *M. tuberculosis* can be detected in a considerable number of skin-biopsy specimens of lobular granulomatous panniculitis. None of the clinical and histologic variables evaluated could accurately predict the results of PCR amplification.—Authors' Abstract

**Bermudez, L. E. and Petrofsky, M.** Regulation of the expression of *Mycobacterium avium* complex proteins differs according to the environment within host cells. Immunol. Cell Biol. **75** (1997) 35–40.

*Mycobacterium avium* is an intracellular organism that can infect a number of cell types such as macrophages and epithelial cells. Each one of these cells represents a different environment that requires specific adaptation from the bacterium. The effect of uptake of *M. avium* and *M. smegmatis* by both human monocyte-derived macrophages in culture for 6 days, and HT-29 intestinal mucosal cell line on the bacterial synthesis of proteins were comparatively examined. Incorporation of [S-35]-methionine by the bacterium was measured at 30 min, 2, 4, and 24 hr after infection. Effect of the uptake by cells was compared with bacteria not exposed to cells and bacteria submitted to different stresses such as heat, hyperosmolarity and acid pH. Uptake of *M. avium* by macrophages triggered the synthesis of 93, 65, 55 and 33 kDa, among other proteins in the bacteria. Between 2 and 4 hr of exposure to the intracellular milieu, a number of additional proteins have their synthesis upregulated, such as 39, 31, 43, 42, 61 and 70 kDa. In contrast, uptake by epithelial cells is associated with the upregulation of 27, 65, 71 and 72 kDa proteins, among others. In this case, exposure to the intracellular environment was associated with expression of a number of proteins that do not vary with time. The results of this study suggest that regulation of the expression of proteins in *M. avium* varies according to the mammalian cell bacteria they are exposed to, and is influenced by the stage of intracellular infection.—Authors' Abstract

**Brown, D. H., Lafuse, W. P. and Zwilling, B. S.** Stabilized expression of mRNA is associated with mycobacterial resistance controlled by Nramp1. Infect. Immun. **65** (1997) 597–603.

Control of innate resistance to the growth of mycobacteria is mediated by a gene termed Nramp1. Although the role of the protein product of Nramp1 in mediating resistance to mycobacterial growth is not

known, the effect of the gene is pleiotropic and it has been suggested that the gene controls macrophage priming for activation. We have found that the functional capacity of macrophages from *Mycobacterium bovis* BCG-susceptible mice can be suppressed by corticosterone, while the function of macrophages from BCG-resistant mice remains unaffected. In this study, we show that corticosterone differentially affects the stability of mRNAs of several recombinant gamma interferon (IFN-$\gamma$)-induced genes. Treatment of macrophages from BCG-susceptible mice with corticosterone accelerates the decay of Nramp1 mRNA. The mRNA of IFN-$\gamma$-induced genes of macrophages from BCG-resistant mice was more stable than the mRNA of macrophages from BCS-susceptible mice in the presence or absence of corticosterone. The results of this investigation suggest that Nramp1 acts by stabilizing the mRNA of genes associated with macrophage activation, thus accounting for the functional differences that have been attributed to these macrophage populations.—Authors' Abstract

**Brown, K. S.** New uses for thalidomide yielding valuable lessons. Scientist **11** (1997) 1.

The good, the bad, the therapeutic: Thalidomide, the teratogenic drug that caused a rash of birth defects 35 years ago, has potential benefit as a therapy for AIDS and leprosy; as several companies work with the Food and Drug Administration to pursue it for these new uses, others are looking to the effort for lessons on developing drugs that cause birth defects.—Author's Abstract

**Brugiere, O., Vokurka, M., Lecossier, D., Mangiapan, G., Amrane, A., Milleron, B., Mayaud, C., Cadranel, J. and Hance, A. J.** Diagnosis of smear-negative pulmonary tuberculosis using sequence capture polymerase chain reaction. Am. J. Respir. Crit. Care Med. **155** (1997) 1478–1481.

Techniques based on the polymerase chain reaction (PCR) can be used to rapidly identify DNA from *Mycobacterium tuberculosis* in clinical samples from patients with tuberculosis, but prior studies evaluating this approach in the diagnosis of paucibacillary forms of pulmonary tuberculosis have reported poor sensitivity and/or specificity. We have developed a procedure in which mycobacterial DNA in crude samples is specifically captured prior to amplification, thereby concentrating the target sequences and removing irrelevant DNA and other inhibitors of the amplification reaction (sequence capture PCR). To evaluate the usefulness of this approach in the diagnosis of paucibacillary forms of pulmonary tuberculosis, sequence capture PCR was performed prospectively on samples of bronchoalveolar lavage fluid from consecutive patients suspected of having pulmonary tuberculosis but for whom three consecutive samples of respiratory secretions were smear negative. Of the 27 patients evaluated, active tuberculosis was diagnosed in nine; sequence capture PCR was positive for all of these patients, including the three for whom all specimens submitted for culture were negative. No positive results were obtained for lavage fluid from the 18 patients for whom the diagnosis of active tuberculosis was subsequently excluded or 25 additional patients undergoing bronchoalveolar lavage for evaluation of other pulmonary problems, even though many of these patients had a history of prior tuberculosis or radiographic evidence of prior tuberculous infection. Paucibacillary forms of pulmonary tuberculosis can be rapidly identified with high sensitivity and specificity using sequence capture PCR performed on samples obtained by bronchoalveolar lavage.—Authors' Abstract

**Castilla, E. E., Ashton Prolla, P., Barreda Mejia, E., Brunoni, D., Cavalcanti, D. P., Correa Neto, J., Delgadillo, J. L., Dutra, M. G., Felix, T., Giraldo, A., Juarez, N., Lopez Camelo, J. S., Nazar, J., Orioli, I. M., Paz, J. E., Pessoto, M. A., Pina Neto, J. M., Quadrelli, R., Rittler, M., Rueda, S., Saltos, M., Sanchez, O. and Schuler, L.** Thalidomide, a current teratogen in South America. Teratology **54** (1996) 273–277.

Thalidomide, mainly used for the treatment of leprosy, is a current teratogen in

South America, and it is reasonable to assume that at present this situation is affecting many births in underdeveloped countries. Moreover, the potential re-marketing of thalidomide for the treatment of a large variety of diseases may extend the problem to the developed world. When the drug is available, the control of its intake during early pregnancy is very difficult since most pregnancies are unintended. The ongoing occurrence of thalidomide embryopathy cases went undetected by the ECLAMC, due to several factors: 1) low populational coverage through this monitoring system; 2) pre-existence of the teratogen with its effects present in both baseline (expected) and monitored (observed) materials; and 3) lack of a defined phenotype to be monitored. Thus, if thalidomide re-enters the market throughout the world, due to the wide range of new applications, occurrence of phocomelia alone might not be sufficient to detect its effects. By a case-reference approach, the ECLAMC registered 34 thalidomide embryopathy cases born in South America after 1965 whose birthplaces correspond to endemic areas for leprosy. Phocomelia was found in 5 of 11 fully described cases. Thus, phocomelia alone is neither specific nor sufficient to serve as a suitable phenotype to survey the teratogenic effects of thalidomide. Therefore, a thalidomide-like phenotype, defined as any bilateral upper and/or lower limb reduction defect of the preaxial and/or phocomelia types, should be included in the routine surveillance of birth defects in all programs.—Authors' Abstract

**Dhople, A. M., Ibanez, M. A. and Poirier, T. C.** Role of iron in the pathogenesis of *Mycobacterium avium* infection in mice. Microbios **87** (1996) 77–87.

Mycobacterial infections are of serious concern to HIV-infected patients, and take a heavy toll of such patients. *Mycobacterium avium* is the most common opportunistic bacterial infection in patients with AIDS. The overload of iron in serum has been implicated in the pathogenicity of a number of bacterial infections. Since iron storage in cells such as macrophages is increased in AIDS, the role of iron as a possible factor in the pathogenesis of *M. avium* infection was examined. Supplementing iron to normal laboratory chow resulted in accelerated *M. avium* infection in mice inoculated earlier the same organism. The bacterial loads in liver, spleen and lungs were approximately 12-fold higher in mice receiving iron supplementation compared with control groups. This is attributed to an increased percentage saturation of iron in the sera of the mice, thus making more iron available for the replication of bacteria. The addition of beef fat to the diet, together with high iron supplementation, further enhanced the infection. Using smaller inocula, mice receiving chow supplemented with high iron and fat developed disseminated *M. avium* infection faster than control mice. The results provide strong evidence that iron may play a major role in the pathogenesis of *M. avium* infection.—Authors' Abstract

**Dubnau, E., Laneelle, M.-A., Soares, S., Benichou, A., Vaz, T., Prome, D., Prone, J.-C., Daffe, M. and Quemard, A.** *Mycobacterium bovis* BCG genes involved in the biosynthesis of cyclopropyl keto- and hydroxy-mycolic acids. Mol. Microbiol. **23** (1997) 313–322.

The resurgence of tuberculosis and the emergence of multidrug-resistant mycobacteria necessitate the development of new antituberculosis drugs. The biosynthesis of mycolic acids, essential elements of the mycobacterial envelope, is a good target for chemotherapy. Species of the *Mycobacterium tuberculosis* complex synthesize oxygenated mycolic acids with keto and methoxy functions. In contrast, the fast-growing *M. smegmatis* synthesizes oxygenated mycolic acids with an epoxy function. We describe the isolation and sequencing of a cluster of four genes from *M. bovis* bacillus Calmette-Guérin (BCG), coding for methyl transferases, and which, when transferred into *M. smegmatis*, allow the synthesis of ketomycolic acid, in addition to an as yet undescribed mycolic acid, hydroxymycolic acid. These oxygenated mycolic acids, unlike the regular mycolic acids of *M. smegmatis*, and similar to the mycolic acids of *M. bovis*, are highly cyclopropanated. Furthermore, there is a perfect

match between the structures of the keto- and the hydroxy-mycolic acids. We propose a biosynthetic model in which there is a direct relationship between these two types of mycolic acid.—Authors' Summary

**Feizabadi, M. M., Robertson, I. D., Cousins, D. V., Dawson, D. J. and Hampson, D. J.** Use of multilocus enzyme electrophoresis to examine genetic relationships amongst isolates of *Mycobacterium intracellulare* and related species. Microbiology **143** (1997) 1461–1469.

As part of a larger study investigating diversity and distribution of *Mycobacterium* spp. in Australia, multilocus enzyme electrophoresis was used to assess genetic relationships at 17 enzyme loci among a collection of reference strains and isolates initially identified on biochemical and other grounds as *Mycobacterium intracellulare* (70), 'X' mycobacteria (10), *M. scrofulaceum* (7), *M. avium* (8) and *M. avium* subsp. *paratuberculosis* (2). Two of the isolates initially identified as *M. intracellulare* were shown to be quite distinct from the others. Both gave negative results in a species-specific DNA probe test, while one was positive by PCR. These results emphasize the uncertainties involved in identifying members of this group. The other *M. intracellulare* isolates formed a cohesive but diverse group, being divided into 48 electrophoretic types (ETs), with a mean genetic diversity of 0.38. Forty-three of these ETs contained only single isolates. There was no clear relationship between the serovar and ET designation. The index of association calculated for *M. intracellulare* was significantly different from zero, suggesting that it is a clonal species. PFGE was also applied to selected isolates from the ETs containing multiple isolates, and some of these could be differentiated further. The strains of *M. scrofulaceum* and 'X' mycobacteria were distinct from *M. intracellulare*, but themselves were highly heterogeneous, with mean genetic diversities of 0.66 and 0.65, respectively. Each of these groups may represent more than one species. *M. avium* strains were distinct from the two *M. avium* subsp. *paratuberculosis* strains, as well as from the other mycobacteria studied.—Authors' Abstract

**Fratazzi, C., Arbeit, R. D., Carini C. and Remold, H. G.** Programmed cell death of *Mycobacterium avium* serovar 4-infected human macrophages prevents the mycobacteria from spreading and induces mycobacterial growth inhibition by freshly added, uninfected macrophages. J. Immunol. **158** (1997) 4320–4327.

*Mycobacterium avium*, an opportunistic pathogen in AIDS patients, replicates in human macrophages and induces programmed cell death (PCD). In this study we examine the effect of freshly added, uninfected macrophages on *M. avium* growth in apoptotic macrophages cultures. Incubation of uninfected autologous macrophages with apoptotic macrophages infected with *M. avium* for 6 hr results in 90% inhibition of bacterial growth. The uninfected macrophages adhere to *M. avium*-infected apoptotic, but not to nonapoptotic *M. avium*-infected macrophages, suggesting a specific interaction between apoptotic and nonapoptotic macrophages. PCD of the host macrophages also prevents the release of intracellular components and the spread of the mycobacterial infection.

Once the apoptotic infected macrophages reach the necrotic stage, mycobacteria and other intracellular material are released; the latter suffice to support extracellular mycobacterial replication. Necrosis of *M. avium*-infected macrophages is significantly augmented by the transglutaminase inhibitors dansylcadaverine and cystamine, indicating that apoptosis of macrophages is dependent on the extent of crosslinking of cell proteins by transglutaminases. Consequently, transglutaminase inhibitors accelerate the release of mycobacteria and intracellular components from the infected macrophages into the medium. These findings indicate that PCD of *M. avium*-infected macrophages is an important defense mechanism, preventing the spread of infection by sequestering the mycobacteria and by contributing to their demise by activation of newly recruited uninfected macrophages.—Authors' Abstract

**Garza Gonzalez, E., Guerrero Olazaran, M., Tijerina Menchaca, R. and Viader Salvado, J. M.** Determination of drug susceptibility of *Mycobacterium tuberculosis* through mycolic acid analysis. J. Clin. Microbial. **35** (1997) 1287–1289.

In the present work a rapid method to determine the susceptibility of *Mycobacterium tuberculosis* to isoniazid and streptomycin by determining levels of mycolic acids by high-performance liquid chromatography (HPLC) was developed. Mycobacterial growth kinetics in the presence and absence of antituberculosis drugs was characterized by evaluating the total area corresponding to mycolic acid peaks (TAMA). Results show a linear relationship between the logarithm of CFU per milliliter and TAMA and show that it is possible to detect growth inhibition of *M. tuberculosis* in the presence of isoniazid or streptomycin by using HPLC in 3 and 4 days, respectively.—Authors' Abstract

**Hernandez Pando, R., Orozco, H., Arriaga, K., Sampieri, A., Larriva Sahd, J. and Madrid Marina, V.** Analysis of the local kinetics and localization of interleukin-1 alpha, tumour necrosis factor-alpha and transforming growth factor-beta during the course of experimental pulmonary tuberculosis. Immunology **90** (1997) 607–617.

A mouse model of pulmonary tuberculosis induced by the intratracheal instillation of live and virulent mycobacteria strain H37Rv was used to examine the relationship of the histopathological findings with the local kinetics production and cellular distribution of tumor necrosis factor-alpha (TNF-$\alpha$), interleukin 1-alpha (IL-1$\alpha$) and transforming growth factor-beta (TGF-$\beta$). The histopathological and immunological studies showed two phases of the disease: acute or early and chronic or advanced. The acute phase was characterized by inflammatory infiltrate in the alveolar-capillary interstitium, blood vessels and bronchial wall with formation of granulomas. During this acute phase, which lasted from 1 to 28 days, high percentages of TNF-$\alpha$ and IL-1$\alpha$ immunostained activated macrophages were observed principally in the interstrium-intralveolar inflammatory infiltrate and in granulomas. Electron microscopy studies of these cells showed extensive rough endoplasmic reticulum, numerous lysosomes and occasional mycobacteria. Double labeling with colloid gold showed that TNF-$\alpha$ and IL-1$\alpha$ were present in the same cells, but were confined to separate vacuoles near the golgi area, and mixed in larger vacuoles near to cell membrane. The concentration of TNF-$\alpha$ and IL-1$\alpha$ as well as their respective mRNAs were elevated in the early phase, particularly at day 3 when the bacillary count decreased. A second peak was seen at days 14 and 21–28 when granulomas appeared and evolved to full maturation. In contrast, TGF-$\beta$ production and numbers of immunoreactive cells were low in comparison with the advanced phase of the disease. The chronic phase was characterized by histopathological changes indicative of more severity (i.e., pneumonia, focal necrosis and extensive interstitial fibrosis) with a decrease in the TNF-$\alpha$ and IL-1$\alpha$ production that coincided with the highest level of TGF-$\beta$. The bacillary counts were highest as the macrophages became large, vacuolated foamy cells, and containing numerous bacilli with immunoreactivity to mycobacterial lipids and lipoarabinomannan (LAM). These macrophages displayed poor and scarce TNF-$\alpha$ and IL-1$\alpha$ immunostaining but still strong immunoreactivity to TGF-$\beta$. These cytokine production kinetics and the spatial relationship between immunostained cells and lung lesions corroborate the important role of TNF-$\alpha$ and IL-1$\alpha$ in the constitution of granulomas and immune protection during the early phase of the infection, and also suggest an important if not primary role for TGF-$\beta$ in the immunopathogenesis of the advanced forms of pulmonary tuberculosis.—Authors' Abstract

**Hines, M. E., Cray, C., Elvinger, F. and Altman, N. H.** Macrophage inhibitory factor-A3 derived from *Mycobacterium avium* serovar 2 inhibits candidacidal activity of murine peritoneal macrophages. Vet. Microbiol. **53** (1996) 295–302.

Macrophage inhibitory factor-A3 (MIF-A3), a fraction derived from *Mycobac-*

*terium avium* serovar 2, inhibited candidacidal activity in macrophages from C57BL/6, C57BL/10, C3H/HeJ and A/J strains of mice. Inhibition of candidacidal activity was demonstrated at MIF-A3 concentrations ranging from 100–400 μg/ml in macrophages without additional stimulators (exception C3H/HeJ macrophages) and in macrophages additionally stimulated with 200 U/ml interferon-gamma, 100 ng/ml phorbol myristate acetate and 0.4 ng/ml *E. coli* lipopolysaccharide from all mouse strains tested. The decreased candidacidal effect produced by MIF-A3 was dose-dependent and appeared greatest in macrophages treated with phorbol myristate acetate and lipopolysaccharide. This effect was neutralized by the addition of goat anti-MIF-A3 antiserum. Macrophages from the Bcg(s) mouse strains (C57BL/6 and C57BL/10) were more sensitive to the effect(s) of MIF-A3 than macrophages from the Bcg′ mouse strains (C3H/HeJ and A/J).—Authors' Abstract

**Kamerbeek, J., Schouls, L., Kolk, A., van Agterveld, M., van Soolingen, D., Kuijper, S., Bunschoten, A., Molhuizen, H., Shaw, R., Goyal, M. and van Embden, J.** Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. **35** (1997) 907–914.

Widespread use of DNA restriction fragment length polymorphism (RFLP) to differentiate strains of *Mycobacterium tuberculosis* to monitor the transmission has been hampered by the need to culture this slow-growing organism and by the level of technical sophistication needed for RFLP typing. We have developed a simple method which allows simultaneous detection and typing of *M. tuberculosis* in clinical specimens and reduces the time between suspicion of the disease and typing from 1 or several months to 1 or 2 days. The method is based on polymorphism of the chromosomal DR locus, which contains a variable number of short direct repeats interspersed with nonrepetitive spacers. The method is referred to as spacer oligotyping or "spoligotyping" because it is based on strain-dependent hybridization patterns of *in vitro*-amplified DNA with multiple spacer oligonucleotides. Most of the clinical isolates tested showed unique hybridization patterns, whereas outbreak strains shared the same spoligotype. The types obtained from direct examination of clinical samples were identical to those obtained by using DNA from cultured *M. tuberculosis*. This novel preliminary study shows that the novel method may be a useful tool for rapid disclosure of linked outbreak cases in a community, in hospitals, or in other institutions and for monitoring of transmission of multidrug-resistant *M. tuberculosis*. Unexpectedly, spoligotyping was found to differentiate *M. bovis* from *M. tuberculosis*, a distinction which is often difficult to make by traditional methods.—Authors' Abstract

**Lee, B. S. J., Wegner, S. A., McGarigle, C. J., Bierer, B. E. and Antin, J. H.** Treatment of chronic graft-versus-host disease with clofazimine. Blood **89** (1997) 2298–2302.

Clofazimine (Lamprene) is an antimycobacterial drug that has antiinflammatory activity in a number of chronic autoimmune skin disorders. We report 22 patients treated with clofazimine for chronic graft-versus-host disease (cGVHD). The initial dose was 300 mg orally in a single daily dose for 90 days. After 90 days, the dose was lowered to 100 mg orally each day and the medication continued indefinitely as tolerated. Treatment courses lasted 7 to 835 days and were generally well tolerated. Gastrointestinal side effects occurred in 8 of 22 patients (36%) and hyperpigmentation was noted in 12 of 22 patients (55%), which resolved upon decrease or discontinuation of the drug. Over 50% of patients with skin involvement, flexion contractures, or oral manifestations achieved complete or partial responses. Seven of 22 patients (32%) were able to reduce other immunosuppressive medications. Thus, clofazimine is safe and has encouraging efficacy in cGVHD, particularly if sclerodermatous skin, joint contractures, or oral manifestations are present. The mechanism by which clofazimine induces a response is unknown, but might be secondary to suppression of alloreactive T-

cell function in cGVHD target organs. Clofazimine deserves further study for the treatment of cGVHD.—Authors' Abstract

**Nguyen, M., Tran, C., Barsky, S., Sun, J. R., McBride, W., Pegram, M., Pietras, R., Love, S. and Glaspy, J.** Thalidomide and chemotherapy combination: preliminary results of preclinical and clinical studies. Int. J. Oncol. **10** (1997) 965–969.

Angiogenesis has been shown to be important in tumor growth and metastasis. Thalidomide, an oral sedative, has recently been found to inhibit angiogenesis. We therefore set out to ask whether thalidomide can be used as therapy for breast cancer. In a mouse model of breast cancer, we found that thalidomide alone did not suppress tumor growth. However, mice treated with thalidomide in combination with cytoxan and adriamycin had significantly smaller tumors than those given the two chemotherapeutic agents alone ($3432 \pm 303$ mm$^3$ versus $4643 \pm 203$ mm$^3$, $p = 0.0005$). We proceeded to administer thalidomide together with chemotherapy to seven breast cancer patients in the context of a Phase I trial. Side effects attributed to thalidomide were minimal, and included constipation and a rash. We concluded that an approach at cancer therapeutics combining an anti-angiogenic agent such as thalidomide with conventional chemotherapy may be feasible and deserves further studies.—Authors' Abstract

**Osaki, M., Adachi, H., Gomyo, Y., Yoshida, H. and Ito, H.** Detection of mycobacterial DNA in formalin-fixed paraffin-embedded tissue specimens by duplex polymerase chain reaction: application to histopathologic diagnosis. Mod. Pathol. **10** (1997) 78–83.

Granuloma is a chronic inflammatory process associated with noninfectious agents or infectious diseases such as tuberculosis. Determination of the causative agent might be occasionally difficult in histopathologic sections. In this study, we examined 60 specimens of granuloma or inflammatory lesions that were originally diagnosed as 51 cases of granulomatous inflammation, 6 of leprosy, and 3 of atypical mycobacteriosis. The diagnoses in the last two categories were made both histologically and clinically. All of the sections and DNA were prepared from formalin-fixed, paraffin-embedded blocks. Histopathologic and immunohistochemical findings were compared with the results of duplex polymerase chain reaction (PCR) using two primers to amplify mycobacterial-common 383-base pair (bp) DNA and *Mycobacterium tuberculosis*-complex-specific 240-bp DNA. Six samples of leprosy and three of atypical mycobacteriosis showed the 383-bp but not the 240-bp band. Among the 51 specimens of granulomatous inflammations, nine showed no band of even the betaglobin, the cases being excluded from this analysis. The 42 specimens of granulomatous inflammation were subdivided into three categories by PCR: 1) 383- and 240-bp positive; 2) 383-bp positive and 240-bp negative; and 3) both negative. Category 1 included 32 specimens (76.2%), being considered as tuberculosis. One specimen was classified into Category 2, indicating possible atypical mycobacterium. Category 3 included nine specimens, composed of five of sarcoidosis and four other agent-induced granulomas, when compared with histologic and clinical findings. These findings indicate that the PCR assay using DNA extracted from paraffin-embedded materials provides useful information to differentiate tuberculosis from other type of granulomas.—Authors' Abstract

**Peirs, P., de Wit, L., Braibant, M., Huygen, K. and Content, J.** A serine/threonine protein kinase from *Mycobacterium tuberculosis*. Eur. J. Biochem. **244** (1997) 604–612.

Genomic DNA sequencing in the vicinity of the pstA-1 gene from *Mycobacterium tuberculosis* allowed us to clone, sequence and identify a gene encoding a 70-kDa protein. The size of the protein was confirmed by *in vitro* coupled transcription/translation. Its *N*-terminal domain shows extensive sequence similarity with the catalytic domain of eukaryotic serine/threonine protein kinases, and the protein was therefore called Mbk (mycobacterial protein kinase). The

deduced amino acid sequence contains two transmembrane segments, which flank a highly repetitive region, suggesting a receptor-like anchoring. The mbk gene was overexpressed in *Escherichia coli* and the gene product (Mbk) was purified as a fusion protein with gluthatione S-transferase. Recombinant Mbk was found to be autophosphorylated on threonine residues and capable of phosphorylating myelin basic proteins from bovine brain and histones from calf thymus on serine residues, both in a manganese-dependent manner. The phosphorylation of myelin basic proteins by Mbk was inhibited by calcium and by staurosporine, a widely used inhibitor of eukaryotic protein serine/threonine kinases. A similar gene was found in *M. bovis* BCG DNA by Southern blot analysis. Its expression was detected in cultures of *M. bovis* BCG by reverse transcriptase/PCR. Although its biological role is unknown, it is the first serine/threonine protein kinase characterized in mycobacteria.—Authors' Abstract

**Placido, R., Mancino, G., Amendola, A., Mariani, F., Vendetti, S., Piacentini, M., Sanduzzi, A., Bocchino, M. L., Zembala, M. and Colizzi, V.** Apoptosis of human monocytes/macrophages in *Mycobacterium tuberculosis* infection. J. Pathol. **181** (1997) 31–38.

Tuberculosis (TB) is still a major health problem, both as a single disease entity and as a cofactor in AIDS. The interaction between macrophage and *Mycobacterium tuberculosis* (MTB) is a critical step in the establishment of an early chronic infection. This study analyzes the capacity of MTB to induce apoptosis in cells obtained by broncho-alveolar lavage (BAL) from patients with reactive pulmonary tuberculosis and from AIDS patients with disseminated pulmonary tuberculosis. Apoptosis was increased three-fold in BAL cells obtained from patients with pulmonary tuberculosis and even more markedly in alveolar macrophages of MTB-infected AIDS patients compared with controls. Apoptosis was analyzed and characterized by propidium iodide (PI) incorporation, terminal deoxy transferase (TDT)-mediated dUTP-biotin nick end labeling (TUNEL), and tissue transglutaminase (tTG) expression. The MTB-macrophage interaction was also investigated *in vitro* by infecting monocyte-derived macrophages (MDM) with MTB (virulent strain H37Rv). The induction of apoptosis by MTB required viable bacteria, was dose-dependent, and was restricted to H37Rv. Infection with either *M. avium* complex (MAC) or HIV-1 and treatment with heat-killed MTB failed to induce apoptosis.—Authors' Abstract

**Portaels, F., Aguiar, J., Fissette, K., Fonteyne, P. A., de Beenhouwer, H., de Rijk, P., Guedenon, A., Lemans, R., Steunou, C., Zinsou, C., Dumonceau, J. M. and Meyers, W. M.** Direct detection and identification of *Mycobacterium ulcerans* in clinical specimens by PCR and oligonucleotide-specific capture plate hybridization. J. Clin. Microbiol. **35** (1997) 1091–1100.

We compared various diagnostic tests for their abilities to detect *Mycobacterium ulcerans* infection in specimens from patients with clinically active disease. Specimens from 10 patients from the area of Zangnanado (Department of Zou, Benin) with advanced, ulcerated active *M. ulcerans* infections were studied by direct smear, histopathology, culture, PCR, and oligonucleotide-specific capture plate hybridization (OSCPH). A total of 27 specimens, including 12 swabs of exudate collected before debridement and 15 fragments of tissue obtained during debridement, were submitted to bacteriologic and histopathologic analysis. The histopathologic evaluation of tissues from all six patients so tested revealed changes typical of those caused by *M. ulcerans* infection.

Five specimens were contaminated, and *M. ulcerans* was cultivated on Lowenstein-Jensen medium from 12 of the remaining 22 (54.5%) specimens. Detection of mycobacteria was performed by PCR, and *M. ulcerans* was detected by OSCPH with a new probe (5′-CACGGGATTCATGTCCTGT-3′) reacting with *M. ulcerans* and *M. marinum*. In 10 of 22 (45.5%) specimens, *M. ulcerans* was identified by PCR-OSCPH. There was no statistically significant difference between the detection of *M. ul-*

*cerans* by culture and by PCR-OSCPH ($p > 0.05$). This is the first demonstration of an amplification system (PCR-OSCPH) with a sensitivity similar to that of culture for the direct and rapid recognition of *M. ulcerans* in clinical specimens. This system is capable of identifying *M. ulcerans*, even in paucibacillary lesions. Our findings suggest that PCR-OSCPH should be used in the quest for the elusive environmental reservoir(s) of *M. ulcerans*.—Authors' Abstract

**Prakken, B. J., van der Zee, R., Anderton, S. M., van Kooten P. J. S., Kuis, W. and van Eden, W.** Peptide-induced nasal tolerance for a mycobacterial heat shock protein 60 T cell epitope in rats suppresses both adjuvant arthritis and nonmicrobially induced experimental arthritis. Proc. Natl. Acad. Sci. U.S.A. **94** (1997) 3284–3289.

Adjuvant arthritis (AA) can be induced in Lewis rats by immunization with mycobacterial antigens. Passive transfer of a T-cell clone recognizing the 180–188 amino acid sequence in mycobacterial heat shock protein 60 (hsp60) was found to induce AA. In the present study, we investigated whether tolerance was obtained for this AA-associated T-cell epitope after intranasal or s.c. administration of a peptide containing this epitope. Two 15-mer peptides containing the mycobacterial hsp60 sequences 176–190 and 211–225 were used; 176–190 contained the T-cell epitope 180–188, which was recognized by the arthritogenic T-cell clone A2b and was the immunodominant hsp60 T-cell epitope after induction of AA, and 211–225 contained a T-cell epitope that was recognized both after induction of arthritis with whole *Mycobacterium tuberculosis* and after immunization with mycobacterial hsp60. In rats treated intranasally or subcutaneously with 176–190 and immunized with mycobacterial hsp60, proliferative responses to 176–190 were reduced. Proliferative responses to 211–225 and to whole mycobacterial hsp60 were not affected. AA was inhibited intranasally in the 176–190-treated rats but not in the 211–225-treated rats. Moreover, intranasal 176–190 led to similar arthritis-protective effects in a nonmicrobially induced experimental arthritis (avridine-induced arthritis). Therefore, tolerance for a disease-triggering, microbial cartilage-mimicking epitope may cause resistance to arthritis irrespective of the actual trigger leading to development of the disease.—Authors' Abstract

**Quinting, B., Galleni, M., Timm, J., Gicquel, B., Amicosante, G. and Frere, J. M.** Purification and properties of the *Mycobacterium smegmatis* $mc^2$ 155 beta-lactamase. FEMS Microbiol. Lett. **149** (1997) 11–15.

The beta-lactamase of *Mycobacterium smegmatis* mc(2)155 has been purified to protein homogeneity. Its *N*-terminal sequence and catalytic properties are similar to those of the beta-lactamase produced by *Mycobacterium fortuitum* D316 and establish this new enzyme as a member of molecular class A.—Authors' Abstract

**Quinting, B., Reyrat, J. M., Monnaie, D., Amicosante, G., Pelicic, V., Gicquel, B., Frere, J. M. and Galleni, M.** Contribution of beta-lactamase production to the resistance of mycobacteria to beta-lactam antibiotics. FEBS Lett. **406** (1997) 275–278.

*Mycobacterium fallax* is naturally sensitive to many beta-lactam antibiotics (MIC < 2 μg/ml) and devoid of beta-lactamase activity. In this paper, we show that the production of the beta-lactamase of *M. fortuitum* by *M. fallax* significantly increased the MIC values for good substrates of the enzyme, whereas the potency of poor substrates or transient inactivators was not modified. The rates of diffusion of beta-lactams through the mycolic acid layer were low, but for all studied compounds the half-equilibration times were such that they would only marginally affect the MIC values in the absence of beta-lactamase production. These results emphasize the importance of enzymatic degradation as a major factor in the resistance of mycobacteria to penicillins.—Authors' Abstract

**Ragno, S., Colston, M. J., Lowrie, D. B., Winrow, V. R., Blake, D. R. and Tas-**

**con, R.** Protection of rats from adjuvant arthritis by immunization with naked DNA encoding for mycobacterial heat shock protein 65. Arthritis Rheum. **40** (1997) 277–283.

Objective. To assess the feasibility of vaccination with naked DNA encoding for mycobacterial heat shock protein 65 (hsp65) in the modulation of experimental arthritis.

Methods. Adjuvant arthritis (AA) was induced in Lewis rats preimmunized, intramuscularly, with a plasmid encoding for hsp65 (pCMV3.65). Clinical scores were recorded for 3–4 weeks, and histologic and radiologic parameters were evaluated. Cellular and antibody reactivity to hsp65 was assessed. The expression of the hsp65 gene was investigated in injected muscles.

Results. The pCMV3.65-treated rats were significantly protected from disease development in comparison with the control groups. This finding correlated with the results of histologic and radiologic examinations of the involved joints. The message for hsp65 was detected in injected muscles. T-cell proliferation and antibodies to this protein were found to be elevated in pCMV3.65-treated rats when compared with both the arthritic control (AA-induced) and the naive (did not receive adjuvant) animals.

Conclusion. We have demonstrated for the first time that naked DNA delivery is feasible in controlling experimental autoimmune disease. Although the actual mechanism of protection has not been fully elucidated.—Authors' Abstract

**Ramakrishnan, L., Valdivia, R. H., McKerrow, J. H. and Falkow, S.** *Mycobacterium marinum* causes both long-term subclinical infection and acute disease in the leopard frog (*Rana pipiens*). Infect. Immun. **65** (1997) 767–773.

*Mycobacterium marinum* grows at an optimal temperature of 33°C, far lower than that for *M. tuberculosis*. Consequently, *M. marinum* infection of mammals is restricted largely to the cooler surfaces of the body, such as the extremities, but it causes a systemic infection in a large number of poikilothermic animals. Here, we describe a laboratory animal model for *M. marinum* disease in the leopard frog (*Rana pipiens*), a natural host species. *M. marinum* causes a chronic granulomatous, nonlethal disease in immunocompetent frogs. Immunosuppression of the frogs with hydrocortisone results in an acute, fulminant, lethal disease. This animal model, in which a spectrum of tuberculosis-like disease can be produced, will be useful for the dissection of the genetic basis of mycobacterial pathogenesis.—Authors' Abstract

**Rhoades, E. R. and Orme, I. M.** Susceptibility of a panel of virulent strains of *Mycobacterium tuberculosis* to reactive nitrogen intermediates. Infect. Immun. **65** (1997) 1189–1195.

Murine bone marrow-derived macrophages were infected with a panel of virulent isolates of *Mycobacterium tuberculosis* including laboratory strains Erdman and H37Rv and various clinical isolates in order to determine the sensitivity of each of these strains to the antimycobacterial activities of macrophage-generated reactive nitrogen intermediates (RNI). All of the *M. tuberculosis* strains grew in murine bone marrow-derived macrophages; however, gamma interferon-primed macrophages limited the initial growth of intracellular bacilli. Some of the mycobacterial strains, including Erdman, were killed over the first 4 days of infection, as evidenced by significant decreases in the number of viable intracellular bacilli determined by a CFU assay. Other mycobacterial strains were not killed during this same period, and some isolates, including CSU 24 and CSU 31, grew steadily in activated macrophages. The accumulation of nitrite on infected monolayers was measured, and it was found that inhibitory levels of RNI did not vary among infections with the different strains.

Nitrite tolerance was determined in a cell-free system for each of the strains in order to compare susceptibilities of the strains to RNI. All of the strains tested were killed by levels of RNI generated by the acidification of 10 mM NaNO2 to pH 6.5 or 5.5, and the strains exhibited a range of tolerance to lower concentrations of RNI. No correlations were observed between such cell-free RNI tolerances and the capacity of bacilli to

resist macrophage RNI-mediated killing. These results indicate that under stringent conditions, RNI can kill *M. tuberculosis*, but that under less harsh, more physiological conditions, the effects of RNI range from partial to negligible inhibition.—Authors' Abstract

**Schroder, K. H., Naumann, L., Kroppenstedt, R. M. and Reischl, U.** *Mycobacterium hassiacum* sp. nov., a new rapidly growing thermophilic mycobacterium. Int. J. System. Bacteriol. **47** (1997) 86–91

A new rapidly growing, scotochromogenic mycobacterium was isolated from urine. This strain is thermophilic (it grows at 65°C), tolerates 5% NaCl, and was unable to utilize any of the sugars tested or citrate or to take up iron. The isolate splits benzamide, urea, nicotinamide, and pyrazinamide and is sensitive to streptomycin, ethambutol, cycloserine, ciprofloxacin, and clarithromycin but resistant to isoniazid, rifampin, and prothionamide. These characteristics clearly place this organism in a new mycobacterial species, which was confirmed by the unique 16S rRNA nucleotide sequence. The high level of similarity between this rapid grower and *Mycobacterium xenopi* is surprising. For this new, rapidly growing, scotochromogenic and thermophilic mycobacterium we propose the name *Mycobacterium hassiacum* sp. nov.—Authors' Abstract

**Selvakumar, N., Kumar, V. and Paramasivan, C. N.** *In vitro* susceptibility of clinical isolates of *Mycobacterium tuberculosis* to cefadroxil—a cephalosporin antibiotic. Indian J. Med. Res. **105** (1997) 58–60.

The bactericidal activity (BA) of cefadroxil, a semisynthetic cephalosporin antibiotic, against *Mycobacterium tuberculosis* H37Rv was studied in Middlebrook 7H9 medium. Cefadroxil showed good BA (average fall of viable counts = $\log_{10}$ 0.32 colony forming units/ml/day) against the log phase culture of *M. tuberculosis* H37Rv. Its minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were found to be 15 μg/ml or less. The MIC of cefadroxil for 29 clinical isolates of *M. tuberculosis* and a laboratory strain, *M. tuberculosis* H37Rv was also determined by agar dilution method using Middlebrook 7H11 agar as a screening procedure. The MIC of cefadroxil was found to be 10 μg/ml or less for *M. tuberculosis* H37Rv and 16 (55.1%) of 29 clinical isolates tested. The MIC for 3 of 10 drug-sensitive and 9 of 19 drug-resistant isolates was 40 or more, a concentration much higher than the peak plasma concentration (28 μg/ml) attained in human beings. The higher MIC observed in 12 of 29 clinical isolates irrespective of their susceptibility pattern requires further studies to assess the usefulness of cefadroxil in the treatment of tuberculosis.—Authors' Abstract

**Sun, X., *et al.*** [Disability of leprosy patients on diagnosing and its related factors.] China Lepr. J. **12** (1996) 222–224. (in Chinese)

Analysis of factors impacting on the degree of their disability on diagnosing showed that knowledge on leprosy they have had, disease duration, the way they were found, their reaction to awareness of having the disease and marriage status were the major ones. Some measures are proposed for adjusting them.—Authors' English Abstract

**Tavares, J. L., Wangoo, A., Dilworth, P., Marshall, B., Kotecha, S. and Shaw, R. J.** Thalidomide reduces tumour necrosis factor-alpha production by human alveolar macrophages. Respir. Med. **91** (1997) 31–39.

Overexuberant production of tumor necrosis factor-alpha (TNF-$\alpha$) by macrophages and other cells is thought to contribute to the development of permanent lung damage in many inflammatory conditions. There is a need for an agent, without the side effects of corticosteroids, which can reduce the production of TNF-$\alpha$ by macrophages activated by disease. This study evaluated the effect of thalidomide on lipopolysaccharide (LPS)-induced TNF-$\alpha$ production by human alveolar macrophages obtained from patients with tuberculosis and a group of other diseases associated with macrophage activation.

Alveolar macrophages obtained by bronchoalveolar lavage from 31 patients (tuberculosis = 12, sarcoidosis = 3, lung cancer = 5, chronic bronchitis = 5, pneumonia = 6) were stimulated with LPS alone or LPS in combination with either thalidomide or dexamethasone. Cell-associated TNF-$\alpha$, as measured by immunochemistry, and TNF-$\alpha$ released by macrophages, as assessed by ELISA, were markedly increased when cells were incubated with LPS ($p < 0.05$), and both were decreased following addition of thalidomide ($p < 0.05$) or dexamethasone ($p < 0.05$) to amounts similar to those observed when macrophages were incubated with medium alone. Similarly, TNF-$\alpha$ mRNA as measured by *in situ* hybridization was increased following incubation with LPS ($p < 0.05$), but this increase was prevented by addition of thalidomide ($p < 0.05$) or dexamethasone ($p < 0.05$). The ability of thalidomide to reduce LPS-induced TNF-$\alpha$ production by alveolar macrophages was the same when cells from patients with tuberculosis (a disease associated with TNF-$\alpha$ production) and cells from patients with the other conditions were compared.

The ability of thalidomide to reduce TNF-$\alpha$ production by human alveolar macrophages from patients with active lung disease suggests that thalidomide and its analogs may have potential as drugs to reduce TNF-$\alpha$ production in disease.—Authors' Abstract

**Tomiyama, T., Kaneko, H., Kataoka, K., Asano, S. and Endo, N.** Rifampicin inhibits the toxicity of pre-aggregated amyloid peptides by binding to peptide fibrils and preventing amyloid-cell interaction. Biochem. J. **322** (1997) 859–865.

Rifampin and its analogs, p-benzoquinone and hydroquinone, inhibited the toxicity of preformed aggregates of human islet amyloid polypeptide, amylin, to rat pheochromocytoma PC12 cells, when preincubated with the aggregated peptide before addition to cell cultures. Immunofluorescence microscopy showed that they prevented the adhesion of amylin aggregates to the cell surface, and this effect was induced probably by their binding to peptide fibrils during preincubation. Other quinone derivatives, i.e., p-methoxyphenol, AA-861 and idebenone, failed to inhibit the toxicity and cell surface adhesion of amylin aggregates. Rifampin analogs also inhibited the toxicity of pre-aggregated amyloid beta 1-42 peptides, suggesting a common toxic mechanism of different amyloid peptides and their therapeutic potential for several amyloidoses.—Authors' Abstract

**Tuerlinckx, D., Vermylen, C., Brichard, B., Ninane, J. and Cornu, G.** Disseminated *Mycobacterium avium* infection in a child with decreased tumour necrosis factor production. Eur. J. Pediatr. **156** (1997) 204–206.

Disseminated atypical mycobacterial infection is essentially reported in cellular immunodeficient children. Cell-mediated immunity, including cytokines like tumor necrosis factor-alpha (TNF-$\alpha$) and gamma interferon as the most important factor allowing control of the dissemination of mycobacterium. We report a child with disseminated *Mycobacterium avium* infection without classical immunodeficiency or HIV infection. Immunological studies revealed a defect of TNF production when the monocytes of the patient were primed with endotoxin (*Escherichia coli*). Conclusion: This patient represents a further case of possible macrophage defect, explaining the susceptibility to intracellular pathogens.—Authors' Abstract

**Zhang, M., Kim, K. J., Iyer, D., Lin, Y. G., Belisle, J., McEnery, K., Crandall, E. D. and Barnes, P. F.** Effects of *Mycobacterium tuberculosis* on the bioelectric properties of the alveolar epithelium. Infect. Immun. **65** (1997) 692–698.

To investigate the hypothesis that *Mycobacterium tuberculosis* penetrates the alveolar epithelium by downregulating its barrier properties, we evaluated the interactions between *M. tuberculosis* and rat alveolar epithelial cell monolayers that are believed to share electrophysiologic properties of the human alveolar epithelium. Nonproteinaceous components of *M. tuberculosis* caused marked declines in electrical resistance and equivalent short-circuit current of the alveolar epithelial cell monolay-

ers, indicating a reduction in the capacity to maintain tight intercellular junctions and to actively reabsorb sodium. *M. tuberculosis* elicited production of tumor necrosis factor-alpha (TNF-$\alpha$) mRNA and protein by alveolar epithelial cells, and the effects of recombinant TNF-$\alpha$ on the bioelectric properties of the alveolar epithelial paralleled those of *M. tuberculosis*. Furthermore, the effects of *M. tuberculosis* on alveolar epithelial resistance were abrogated by neutralizing anti-TNF-$\alpha$ antibodies. These results indicate that *M. tuberculosis* elicits production of TNF-$\alpha$, which in turn reduces the bioelectric barrier properties of the alveolar epithelium. These findings provide insight into potential mechanisms by which *M. tuberculosis* establishes infection and disease in the lung.—Authors' Abstract

**Zhu, J., Bai, X. F., Mix, E., van der Meide, P. H., Zwingenberger, K. and Link, H.** Thalidomide suppresses T- and B-cell responses to myelin antigen in experimental allergic neuritis. Clin. Neuropharmacol. **20** (1997) 152–164.

The effects of thalidomide and, for reference, dexamethasone on T- and B-cell functions were assayed *in vitro* in Lewis rats with experimental allergic neuritis induced by active immunization with bovine peripheral nerve myelin (BPM) and complete Freund's adjuvant. Thalidomide and dexamethasone at the concentration ranges $10^{-5}$–$10^{-7}$ g/ml and $4 \times 10^{-5}$–$4 \times 10^{-9}$ g/ml, respectively, both inhibited phytohemagglutinin (PHA)- and BPM-induced T-cell proliferation as well as levels of PHA- and BPM-reactive interferon (IFN)-gamma-secreting cells, reflecting the suppression of Th1-like cells. The effect of dexamethasone was most pronounced on PHA-induced T-cell proliferation and IFN-gamma secretions; whereas the effect of thalidomide was most pronounced on BPM-induced T-cell proliferation and IFN-gamma secretion. Thalidomide reduced the B-cell responses to both BPM and *Mycobacterium tuberculosis* purified protein derivative, but to a lesser extent than dexamethasone. The *in vitro* design described could be useful to evaluate compounds with putative immunomodulatory activities. The inhibitory effects of thalidomide on autoantigen-induced Th1-cell functions may warrant the use of this substance in T-cell-mediated autoimmune diseases.—Authors' Abstract