CURRENT LITERATURE

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

General and Historical

Britton, W. J. and Hargrave, J. C. Leprosy in the Tropics and Australia. Med. J. Aust. **159** (1993) 326–330.

Leprosy is no longer the feared disease of the past, but it still causes significant morbidity in countries where it is endemic, and it is estimated that up to 2500 million people are at risk of contracting the disease. Control with dapsone monotherapy over the past 20–30 years has been effective in some countries but secondary and, more recently, primary drug resistance is now widespread. This situation prompted the development of multidrug therapy regimens which are proving highly effective. Here the natural history and clinical features are described, as well as the history and impact of leprosy in Australia. Early diagnosis and the current multidrug regimens offer hope for long-term control of the disease.—Authors' Abstract

Hernandez Angulo, M., Valdes Portela, A. and Diaz, M. E. [The history of the treatment of leprosy in Cuba.] Rev. Leprol. Fontilles **19** (1993) 191–197. (in Spanish)

A historical investigation of the evolution of the treatment of leprosy in Cuba since the colonial period to the present moment is reported.—Authors' English Summary

Maeda, M. [Present situation on leprosy rehabilitation activity in China—a brief report on Japan-China Leprosy Rehabilitation Symposium.] Jpn. J. Lepr. **62** (1993) 41–44. (in Japanese)

Japan-China Leprosy Rehabilitation Symposium was held at Suzhou City, China, from 6 to 7 October in 1992. Twenty-four subjects presented by Japanese and Chinese participants were discussed in full. And, we could promote mutual understanding on the problems of leprosy rehabilitation. The well-planned and energetic activities on leprosy control in China are reported in this paper.—Author's English Abstract

Chemotherapy

Avelleira, J. C., Marques, A. B., Viana, F. R. and Andrade, V. L. G. [Efficacy of MDT for the treatment of paucibacillary hanseniasis patients—preliminary results.] Hansenol. Int. **14** (1989) 107–111. (in Portuguese)

The evaluation of the efficaciousness of the MDT regimens recommended by WHO to the hansenian paucibacillary patients is carried out mainly by the suitable follow up of patients after therapeutic discharge. The criterion for inclusion of patients as paucibacillary ones is another point of importance. The authors, based on the follow up of 66 patients who completed treatment and in the absence of relapses until the moment, advise that together with the clinical classification it should be considered factors like: the Mitsuda test, the number of lesions and the bacilloscopy result.—Authors' English Abstract

Carranzana Hernandez, G. B. [Completion of MDT in leprosy, City of Camagüey, Cuba, 1992.] Rev. Leprol. Fontilles **19** (1993) 153–158. (in Spanish)

The results of the multidrug therapy (MDT) in the 255 cases of leprosy registered in Camagüey City, Cuba, in 1992 according to the Program of Control applied since

1989: The number of multibacillary patients is 199 (84.4%) and the paucillary total 26 (11.8%); 21.8% of the prevalence is on treatment and the rest (78.2%) on post-treatment observation. Approximately 98.0% of the cases on treatment with MDT present good compliance and 93.8% of the patients on observation received MDT. The side effects that prevented the administration of MDT of the rest of the patients are discussed.—Author's English Summary

Coleman, M. D. and Jacobus, D. P. Reduction of dapsone hydroxylamine to dapsone during methaemoglobin formation in human erythrocytes *in vitro*. 2. Movement of dapsone across a semipermeable membrane into erythrocytes and plasma. Biochem. Pharmacol. **46** (1993) 1363–1368.

We have used an *in vitro* two-compartment model to investigate the ability of dapsone, formed by erythrocyte-mediated detoxification of its hydroxylamine metabolite, to escape the cells and cross a semipermeable membrane into both plasma and other erythrocytes. Both diethyl dithiocarbamate (DDC)-treated and untreated erythrocytes were incubated with dapsone hydroxylamine and dialyzed against either fresh cells or plasma. Methemoglobin was predominantly detectable in compartment A although the presence of low levels of methemoglobin in compartment B indicated that the hydroxylamine itself had crossed the membrane. In contrast to methemoglobin disposition, recovery of dapsone was higher ($p < 0.05$) in compartment B compared with A for all three treatment groups at 30 and 60 min, but not at the remaining time points. Regression analysis of the cumulative recovery of dapsone over 150 min in all three treatment groups for both compartments A and B showed correlation coefficients close to unity. In compartment A, analysis of the mean slopes of the regression lines indicated that, overall, significantly more dapsone was recovered from group 1 (erythrocytes, hydroxylamine and DDC dialyzed against untreated red cells) compared with group 3 (erythrocytes and hydroxylamine dialyzed against plasma) (0.22 ± 0.05 vs 0.09 ± 0.005; $p < 0.025$). Also in compartment A, significantly more dapsone was recovered from group 2 (erythrocytes and hydroxylamine dialyzed against untreated red cells) compared with group 3 (erythrocytes and hydroxylamine dialyzed against plasma: 0.16 ± 0.02 vs 0.09 ± 0.005). In compartment B, dapsone recovery was significantly greater in group 1 (erythrocytes, hydroxylamine and DDC dialyzed against untreated red cells; slope of regression line: 0.59 ± 0.05) compared with group 2 (erythrocytes and hydroxylamine dialyzed against untreated red cells; slope of line: 0.28 ± 0.02, $p < 0.005$). In addition, dapsone recovery was significantly greater in group 1 (0.59 ± 0.05) compared with group 3 (erythrocytes and hydroxylamine dialyzed against plasma; 0.21 ± 0.02, $p < 0.005$). Dialysis of erythrocytes with dapsone itself over 120 min caused no detectable methemoglobin formation. The process of erythrocyte-mediated dapsone formation from its hydroxylamine may feasibly occur *in vivo* and contribute to the systemic persistence and therapeutic effect of dapsone.—Authors' Abstract

Cornwall, J., Cameron, G. and Ellis-Pegler, R. B. The effects of World Health Organization chemotherapy on imported leprosy in Auckland, New Zealand, 1983–1990. Lepr. Rev. **64** (1993) 236–249.

Between January 1983 and December 1990 in Auckland, New Zealand, 87 patients [28 paucibacillary disease (PBD) and 59 multibacillary disease (MBD)] commenced WHO multidrug therapy (MDT). All were immigrants from the Pacific Islands (65) or Asia (22). A total of 57 patients had already received non-WHO regimens, some continuously, but often intermittently, for many years; 30 patients received WHO MDT only. By December 1990, 50 had completed treatment, with 1 relapse and 1 late reaction, both in patients with PBD treated with WHO MDT only. There have been no relapses in those treated with WHO MDT after prior leprosy treatment.

In those with MBD, type 2 leprosy reactions were less common (16%) in those treated only with WHO MDT than in those treated continuously before 1983 with older regimens (64%). Type 1 leprosy reactions occurred in about 20% of both these groups. The bacterial index fell faster in those who

had had a prolonged prior treatment beginning WHO MDT than in those starting WHO MDT as their initial leprosy chemotherapy. Overall we found WHO MDT was well accepted and the compliance good, but 13 patients (15%) left Auckland before treatment was completed and 6 (7%) during follow up.—Authors' Summary

Denis, A. and Moreau, N. J. Mechanisms of quinolone resistance in clinical isolates: accumulation of sparfloxacin and of fluoroquinolones of various hydrophobicity, and analysis of membrane composition. J. Antimicrob. Chemother. **32** (1993) 379–392.

The accumulation of sparfloxacin and three other fluoroquinolones of decreasing hydrophobicity, pefloxacin, ofloxacin, and ciprofloxacin, into randomly chosen fluoroquinolone-sensitive and -resistant clinical isolates of bacteria (four Enterobacteriaceae, one *Pseudomonas aeruginosa* and one *Staphylococcus aureus*) was measured. There was good correlation between the concentration of drug that inhibited early DNA synthesis in whole cells and the MIC values of the same drug regardless of organism sensitivity or the quinolone. A decrease in permeability in resistant strains compared with sensitive, due to a modification of outer membrane proteins, was often involved in resistance but in all cases other mutations may also be involved, explaining the relatively high level of resistance of the strains. In two resistant strains the DNA gyrase was purified and found to be resistant to inhibition by quinolones. The use of quinolones of differing hydrophobicity emphasized the importance of this property in the uptake of quinolones by bacterial cells, and provided evidence that sparfloxacin used both porin and the self-promoted uptake pathway for its uptake.—Authors' Abstract

Jacob, A. J. W., Rajendran, A., Menezes, J. and Vaz, M. Dapsone induced motor polyneuropathy. Southeast Asian J. Trop. Med. Public Health **23** (1993) 341–343.

A report of a case in a 17-year-old man in India on multidrug treatment for leprosy.—D. N. J. Lockwood (Trop. Dis. Bull.)

Lee, K. H., Shin, J. G., Chong, W. S., Kim, S., Lee, J. S., Jang, I. J. and Shin, S. G. Time course of the changes in prednisolone pharmacokinetics after co-administration or discontinuation of rifampin. Eur. J. Clin. Pharmacol. **45** (1993) 287–289.

We have investigated changes in the pharmacokinetics of prednisolone caused by co-administration or discontinuation of rifampin. Serial IV pharmacokinetic studies of prednisolone (1 mg/kg) in groups of 3 patients over a 1 month period of rifampin co-treatment or after its withdrawal revealed significant changes in the area under the curve, the total clearance, the non-renal clearance and the half-life. The changes in the pharmacokinetic parameters reached a 1.5- to 2-fold plateau after 2 weeks and the half-maximal effect was attained within 5 days. Neither the volume of distribution nor the protein binding of prednisolone were significantly altered.—Authors' Abstract

Revankar, C. R., Gupta, N., Sorensen, B. H., Naik, S. S. and Multicentre Study Group. Further observations on MDT blister-calendar packs in vertical leprosy eradication programmes—a multicentre study (Phase II). Lepr. Rev. **64** (1993) 250–254.

To improve operational efficiency as well as to improve patient compliance in leprosy programs, DANIDA introduced blister-calendar packs (BCP) to deliver MDT in four MDT districts in India in 1987. An objective study (Phase II) involving 343 patients in a trial group (BCP group) and 253 patients in a control group (loose drug group) showed no significant difference in compliance rates for self-administered doses between the two groups. Hence, while assessing the use of BCPs in leprosy programs, other operational benefits such as safe storage, easy transportation, easy drug accounting and safe preservation at home are to be considered. These aspects were followed up from Phase I of the study.—Authors' Summary

Salafia, A. and Chauhan, G. Clofazimine crystals and ceroid bodies in nerve; a case report. Rev. Leprol. Fontilles **19** (1993) 73–76.

Clofazimine is one of the most widely used drugs in leprosy because of its antibacteriostatic and antiinflammatory activity. Deposition of clofazimine has been reported in various organs and body tissues; however, the authors think the deposition of clofazimine crystals in nerve tissue, as far as we know, has never been reported.—Authors' Summary

Schrenzel, J., Dayer, P., Leemann, T., Weidekamm, E., Portmann, R. and Lew, D. P. Influence of rifampin on fleroxacin pharmacokinetics. Antimicrob. Agents Chemother. **37** (1993) 2132–2138.

Staphylococcus aureus infections have been successfully treated in animal models with the combination of fleroxacin and rifampin. We studied the influence of rifampin, a potent cytochrome P-450 inducer, on the pharmacokinetics and biotransformation of fleroxacin in 14 healthy young male volunteers. Subjects were given 400 mg of fleroxacin orally once a day for 3 days to reach steady state. After a wash-out period of 2 days, the same subjects received 600 mg of rifampin orally once daily for 7 days. On days 5 to 7 of rifampin treatment, 400 mg of fleroxacin was again administered once daily. Concentrations of fleroxacin as well as its two major urinary metabolites, N-demethyl- and N-oxide-fleroxacin, in plasma and urine were determined by reverse-phase high-performance liquid chromatography. The extent of hepatic enzyme induction by rifampin was confirmed by a significant increase of 6-beta-hydroxycortisol urinary output from 160.8 ± 41.4 to 544.8 ± 120.7 μg/4 hr. There were no significant changes in the peak fleroxacin concentration in plasma (6.3 ± 1.2 versus 6.2 ± 1.9 mg/liter), time to maximum concentration of fleroxacin in plasma (1.1 ± 0.9 versus 1.3 ± 1.1 hr), or renal clearance (58.3 ± 16.4 versus 61.9 ± 19.2 ml/min). The area under the curve (AUC) (71.4 ± 15.8 versus 62.2 ± 13.7 mg · hr/liter) and the terminal half-life of fleroxacin (11.4 ± 2.2 versus 9.2 ± 1.1 hr) decreased ($p < 0.05$), while the total plasma clearance increased from 97.7 ± 21.6 to 112.3 ± 25.8 ml/min ($p < 0.01$). Despite being statistically significant, this 15% increase in total plasma clearance does not appear to be clinically relevant. Metabolic clearance by N demethylation was increased (6.9 ± 2.4 versus 12.5 ± 3.2 ml/min; $p < 0.01$); whereas clearance by N oxidation did not change (5.8 ± 1.1 versus 5.8 ± 1.5 ml/min). Fleroxacin elimination was slightly increased (about 15%) through induction of metabolic clearance to N-demethyl-fleroxacin. Since fleroxacin levels remained above the MIC for 90% of the tested isolates of methicillin-susceptible *S. aureus* for at least 24 hr, dose adjustment does not appear necessary, at least for short-term treatments.—Authors' Abstract

Uetrecht, J. P., Shear, N. H. and Zahib, N. N-Chlorination of sulfamethoxazole and dapsone by the myeloperoxidase system. Drug Metab. Dispos. **21** (1993) 830–834.

It is known that activation of neutrophils or monocytes leads to the formation of hydrogen peroxide and the release of myeloperoxidase (MPO). We found that sulfamethoxazole was chlorinated by the combination of MPO, hydrogen peroxide, and chloride. The product, N-chlorosulfamethoxazole, is reasonably stable but reacts rapidly with a variety of compounds. The same product was formed by the reaction between sulfamethoxazole and hypochlorous acid, and dapsone was also N-chlorinated by the MPO system or hypochlorous acid. Although N-chlorination was not observed when sulfamethoxazole or dapsone was incubated with activated neutrophils, this is presumably because the chloramine product reacted rapidly with the cells. When radiolabeled sulfamethoxazole was incubated with activated neutrophils, covalent binding was observed. When radiolabeled sulfamethoxazole was incubated with MPO and hydrogen peroxide in the presence of albumin, covalent binding to the albumin occurred. Although binding to albumin occurred in the absence of chloride, it was increased by the presence of chloride. This suggests that N-chlorosulfamethoxazole may be one of several reactive metabolites of sulfamethoxazole that covalently bind to neutrophils. We suspect that covalent binding of arylamine drugs, such as sulfamethoxazole, to activated leukocytes is responsible for some of the adverse reactions associated with these drugs, especially ad-

verse reactions that involve leukocytes such as agranulocytosis or drug-induced lupus.—Authors' Abstract

Van Rensburg, C. E. J., Durandt, C., Garlinski, P. J. and O'Sullivan, J. F. Evaluation of the antineoplastic activities of the riminophenazine agents clofazimine and B669 in tumor-bearing rats and mice. Int. J. Oncol. **3** (1993) 1011–1013.

The riminophenazine agents clofazimine and its analog B669 displayed antitumor activity at 30 mg/kg/day in benzo[a]pyrene (BP) induced sarcomas of mice as well as dimethylbenz-anthracene (DMBA)-induced rat mammary tumors. No hematological toxicity of these drugs at doses up to 60 mg/kg/day for 1 month was observed. This is the first study to document *in vivo* antineoplastic activities of clofazimine and B669.—Authors' Abstract

Walash, M. I., Belal, F., Metwally, M. E. and Hefnawy, M. M. Spectrophotometric determination of rifampin in the presence of its degradation products in pharmaceutical preparations. Analyt. Lett. **26** (1993) 1905–1917.

The determination of rifampin in the presence of its main degradation products, 3-formyl rifampin and rifampin quinone using two spectrophotometric methods is described. Both Glenn's method and first derivative spectrophotometry were successfully adopted. No preliminary separation steps were required in either case. Both methods gave accurate and reproducible results for the determination of the drug in dosage forms. The percentage recoveries ranged from 99.33% ± 0.63 to 100.2% ± 0.44. The proposed methods are more simple and rapid than other existing methods and can be readily adopted in a control laboratory.—Authors' Abstract

Waters, M. F. R. Leprosy 1962–1993. 1. Chemotherapy of leprosy—current status and future prospects. Trans. R. Soc. Trop. Med. Hyg. **87** (1993) 500–503.

The introduction of multidrug therapy (MDT) by the World Health Organization in 1982 has proved to be the most important advance in the management and control of leprosy since the first use of the sulfone drugs 40 years earlier. For the first time, the number of registered leprosy cases has shown a decline from a peak of 5.37 million in 1985 to 3.1 million in February 1992. The two standard MDT regimens have proved simple to apply in most parts of the world, are relatively cheap, generally acceptable, and have shown remarkably few toxic side effects. Nevertheless, difficulties have arisen in distinguishing between multibacillary and paucibacillary leprosy, especially when skin smears are of poor quality. Relapses in paucibacillary leprosy have proved difficult to distinguish from late reversal reactions. In multibacillary leprosy, the duration of treatment, 2–10 years in lepromatous leprosy, is a source of difficulty, and in addition light-skinned patients dislike the skin discoloration caused by clofazimine, for fear that their diagnosis might be discovered. The discovery that three different groups of drugs are highly bactericidal for the leprosy bacillus, although not so rapidly bactericidal as rifampin, raises the possibility of having simplified, shorter, or better supervised regimens in the future as second generation MDT. These drugs include the 4-fluoroquinolones, pefloxacin, ofloxacin and sparfloxacin, the tetracycline minocycline, and the macrolide clarithromycin. Finally, in low-prevalence areas it is opportune to consider chemoprophylaxis and immunoprophylaxis for child contacts of lepromatous patients.—Authors' Abstract

Clinical Sciences

Achenbach, R. S., Kolodny, L. P. and Tiscornia, J. E. [Mouth involvement in two cases of lepromatous leprosy.] Rev. Argent. Dermatol. **74** (1993) 105–108. (in Spanish)

Two cases of lepromatous leprosy with mouth involvement are reported. One of them showed infiltrated lesions and ulcerated lepromas on hard palate while the other one exhibited lepromas on lips, tongue,

uvula, hard palate, supraglottic region and vocal cords. Mucous involvement in lepromatous leprosy is quite frequent and should call not only the attention of dermatologists for whom diagnosis might be obvious but as well the attention of dentists, stomatologists and general practitioners. Such cases are always erroneously or not at all diagnosed since they are seen in patients with longstanding evolution.—Authors' English Summary

Álvarez Domínguez, A., Hernández Angulo, M. and Cordero Rojas, N. Study of some trace elements in leprosy. Rev. Leprol. Fontilles **19** (1993) 143–152.

Serum levels of zinc (Zn), copper (Cu), magnesium (Mg), and calcium (Ca) were determined by atomic absorption spectrophotometry in patients with lepromatous, dimorphous and tuberculoid leprosy. Zn levels were lower in all three groups of patients compared to controls ($p < 0.01$). Copper levels were higher than those of the controls ($p < 0.01$). There were no differences in serum Mg and Ca values between the patients and the controls.—From Authors' Summary

Arruda, M. S. P., Roslindo, N. C., Fleury, R. N. and Nogueira, M. E. S. [Immunologic evaluation of hanseniasis patients with leukoplakia.] Hansenol Int. **16** (1993) 23–28. (in Portuguese)

Twelve lepromatous patients with leukoplakia were immunologically evaluated to determine whether the immune system participated in the manifestations of the leukoplakia. The test used does not show immunologic abnormalities in these patients. Our results do not support the hypothesis of specific immune involvement in the pathogenesis of leukoplakia in the mouth cavity.—Authors' English Abstract

Azulay, R. D. An induction of the late hypersensitivity reaction to DNCB in patients with different clinical forms of hanseniasis in Brazil. Hansenol. Int. **14** (1989) 3–5.

One-hundred-twenty Brazilian patients with several forms of hanseniasis were tested with DNCB. The results were the following: 1) the sensitization of patients with hanseniasis to DNCB was lower than that seen in the general population; 2) the sensitizations of the borderline and virchowian forms of hanseniasis were lower than those seen in the indeterminate and tuberculoid forms.—Author's Abstract

Bobhate, S. K., Madankar, S. T., Parate, S. N., Choudhary, R. K. and Kumbhalkar, D. T. Malignant transformation of plantar ulcers in leprosy. Indian J. Lepr. **65** (1993) 297–303.

Malignant transformation of plantar ulcers in leprosy is not uncommon. The apparent rarity of these neoplasms could be because many observed cases are not reported. To determine the extent of the problem, 133 consecutive cases of plantar ulcers seen over 2 years were studied clinically as well as histologically. Plantar ulcers were more common in the distal third of the foot (64.67%) but malignant transformation was seen more often in plantar ulcers of the proximal third of the foot (64.29%). Malignant transformation was more common in plantar ulcers of long duration. Histologically, most of the lesions were benign, being instances of pseudo-epitheliomatous hyperplasia (57.89%) or atypical pseudo-epitheliomatous hyperplasia (13.53%). However, squamous cell carcinoma was observed in 10.53% of cases. Thus, it may be that more cases with this complication will be detected if it is borne in mind that malignant change may be encountered in such ulcers.—Authors' Abstract

Bwire, R. and Kawuma, H. J. S. Human immunodeficiency virus and leprosy—type 1 reactions, nerve damage and steroid therapy: "a case report." Lepr. Rev. **64** (1993) 267–269.

In this study a 28-year-old female with both BL leprosy and HIV infections is discussed. Her clinical progress was followed until she completed MDT. During this period she developed recurrent reactional episodes, nerve damage and intercurrent illnesses—some of which might have been due to steroids.—Authors' Summary

Ceballos, A., Urquía, M., Rodríguez-Archilla, A., Gómez-Moreno, G. and Ceballos, L. [Oral status of a Hansen's population in Spain.] Rev. Leprol. Fontilles **19** (1993) 167–177. (in Spanish)

We studied the oral status of 37 patients affected by leprosy coming from Hospital San Francisco de Borja (Fontilles). The index DMFT [an index of caries] of the population was 15.56 and index CPITN [an index of peridontal disease] was 2. Nonspecific oral lesions and typical lesions of the mean age of the population studied were observed. Chemical gustometry showed a perception disturbance of the sweet taste, with detection thresholds higher than the control population, maintaining conserved values in the rest of the tastes.—Authors' English Summary

Cohen, P. R. and Kurzrock, R. Benign rheumatoid nodules in a woman with chronic lymphocytic leukemia and borderline lepromatous leprosy. Ann. Rheum. Dis. **52** (1993) 685–688.

A 66-year-old woman with chronic lymphocytic leukemia and borderline lepromatous leprosy who presented with subcutaneous elbow nodules, which were at first suspected to represent either progression of her hematological disease or leprosy, is described. The clinical characteristics of our patient and previous reports of another 24 subjects with adult onset benign rheumatoid nodules are reviewed. Biopsy of the patient's subcutaneous lesion disclosed the histopathology of a rheumatoid nodule; serological and clinical evaluations for rheumatoid arthritis and other rheumatoid-nodule-associated systemic diseases were negative. Adult onset benign rheumatoid nodules are clinically and histologically identical to those found in patients with rheumatoid arthritis. They often appeared in women during their 20s, frequently resolved spontaneously or were adequately treated by excision, and recurred in about one third of patients. The lesions were located in the ocular adnexa in 60% of patients. The most common lesional sites in patients with nonocular benign rheumatoid nodules were the elbows, feet, and knees. None of these patients subsequently developed rheumatoid arthritis or other rheumatoid-nodule-associated diseases during follow-up periods of as long as 20 years. The appearance of subcutaneous nodules is often the harbinger of an associated systemic disorder. Although benign rheumatoid nodules occur infrequently in adults, they should be considered in the differential diagnosis of new nodular lesions.—Authors' Abstract

Dhar, S., Sharma, V. K. and Kaur, S. Facial, glossopharyngeal, vagus and hypoglossal nerve palsy in a case of lepromatous leprosy. Indian J. Lepr. **65** (1993) 333–336.

Involvement of the central nervous system (CNS) is extremely rare in leprosy. There is apparently very little stress in the literature on cranial nerve affection except trigeminal (V) and facial (VII) nerves. However, involvement of olfactory (I), optic (II) and vestibulocochlear (VIII) nerves also has been reported. We report here a case of lepromatous leprosy with facial (VII), glossopharyngeal (IX), vagus (X) and hypoglossal (XII) nerve involvement. To the best of our knowledge, this is the first report of this kind.—Authors' Abstract

Goto, M., Suzuki, M., Kitajima, S. and Imaizumi, M. [Changes and present status of a Japanese National Leprosarium—analysis of smear positive rate and relapse in Hoshizuka-Keiaien.] Jpn. J. Lepr. **62** (1993) 1–12. (in Japanese)

Changes in the clinical features of leprosy in a Japanese National Leprosarium, Hoshizuka-Keiaien, during 20 years (1972–1991) was studied by analyzing clinical records: a) the slit-skin smear positive rate among lepromatous and borderline cases increased from 16.3% (1972) to 28.8% (1981) and then declined to 3% (1991). b) Relapse was 4.25 cases per annum among 817 patients (0.52% per annum). Relapse of lepromatous leprosy (0.42% per annum) is decreasing, and borderline or neuritic relapse (0.12% per annum) of previously lepromatous cases is the major feature in recent years. c) In lepromatous relapse cases, it took 3.5 years on the average to become smear negative again, but this duration is shortening in recently relapsed cases. d) The number of erythema nodosum leprosum cases is remarkably decreased, but iridocyclitis is still observed. e) 85% of inpatients in 1991 are classified as

clinical cure (Japanese criteria 1989). By virtue of the advances in chemotherapy and the aging of the inpatients (average age 68 years), geriatric diseases, instead of leprosy, are becoming the major problems in Japanese National Leprosaria.—Authors' English Abstract

Kirsztajn, G. M., Nishida, S. K., Silva, M. S., Ajzen, H. and Pereira, A. B. Renal abnormalities in leprosy. Nephron **65** (1993) 381–384.

We have evaluated laboratory and clinical manifestations of renal disease in 96 patients with leprosy, looking for a sensitive and early marker for detection and possibly follow-up of nephropathy in these patients. Microscopic hematuria was observed in 21.9% of the cases (with dysmorphic erythrocytes in 71.4% of them). Abnormal microalbuminuria and urinary beta2-microglobulin were found in 15.8% and 19.8% of the cases, respectively. We have observed a high frequency of hematuria, abnormal microalbuminuria and elevation of urinary beta2-microglobulin in these patients still with normal serum creatinine.—Authors' Abstract

Martins, M. C., Palaci, M., Ueki, S. Y. M., Ferrazoli, L., Sato, D. N., Ichikawa, T. and Costa, H. C. [Fluorescence microscopy as a method of performing the bacilloscopic examination in hanseniasis.] Hansenol. Int. **16** (1991) 29–34. (in Portuguese)

In order to compare the fluorescent microscopy and Ziehl-Neelsen methods for detection of acid-fast bacilli in dermal fluid and pulp from the tiny wound of leprosy patients, 355 smears were examined. Both methods have given 98.71% of adjusted concordance (kappa). However, in relation to the bacterial index (Ridley) we found 55% of discordant results. This could be explained by the fact that in the fluorescent method the count of bacilli per clump has been higher.—Authors' English Abstract

Miko, T. L., Lemaitre, C. and Kinfu, Y. Damage and regeneration of peripheral nerves in advanced treated leprosy. Lancet **342** (1993) 521–525.

Despite the rapidly falling prevalence of leprosy, the disability and handicap resulting from loss of protective sensation, due to irreversible nerve damage, will remain a huge medical problem for many years. To elucidate the location and consequences of permanent nerve damage in treated leprosy, a prospective study involving nine patients who underwent leg amputation was conducted. Full-length nerves dissected from amputated legs were studied with histological and immunohistochemical methods. Our main findings were that: in both lepromatous and tuberculoid leprosy nerve damage increased distally, culminating in total destruction of dermal nerves and sensory nerve endings; after the therapy-related decrease of inflammation large-scale nerve regeneration took place; and that regenerating axons persisted for decades and in tuberculoid leprosy they might reach the subcutaneous fat of the plantar skin.

We conclude that nerve regeneration was blocked by fibrous replacement of the distal-most nerves and nerve endings, and that the theoretical basis of nerve grafting in leprosy is in need of further clarification. In some patients, autologous transplantation of skin flaps, probably irrespective of the duration of loss of sensation, might help in regaining protective sensation.—Authors' Abstract

Mishra, B., Malaviya G. N., Girdhar, A., Husain, S. and Girdhar, B. K. Trigeminal neuralgia—a presenting feature of facial leprosy. Lepr. Rev. **64** (1993) 255–258.

Trigeminal neuralgia is a well-recognized clinical entity. However, it has not been reported to mimic leprosy or vice versa. Of the 3 cases reported here, 2 initially presented with neuralgic symptoms similar to that seen in trigeminal neuralgia and later developed borderline lesions on the face. The third case demonstrated a tingling sensation along with firm and palpable supraorbital nerve (a branch of trigeminal nerve), and a very early skin lesion on the face pointed to the need to consider the neuritic type leprosy before concluding the final diagnosis of a disease like trigeminal neuralgia which calls for a different therapeutic approach.—Authors' Summary

Prabhakar, M. C., Appa Rao, A. V. N., Krishna, D. R., Ramanakar, T. V., Bhaskar Rao, P. G. and Narsimha Reddy, K. New approach to curb the transmission of leprosy. Hansenol. Int. **14** (1989) 6–13.

The effect of local treatment of the nose of lepromatous type of patients with different formulations of rifampin in nasal drops/sprays was investigated in a large number of patients. The preparations were either sprayed or instilled into the nostrils after flushing the nostrils with normal saline at 37°C. It was observed that 10 mg/ml of rifampin was effective in reducing the BI and MI to zero in the nose in 7 days in a majority of the patients. No untoward effect was seen in any of the patients. It is suggested that nasal sprays/drops may be able to prevent the transmission of hanseniasis, since the nose is recognized to be an important portal of exit of *Mycobacterium leprae.* Further, when rifampin drops/sprays are used as soon as the diagnosis is made, the nasal deformity may be prevented. It is believed that local treatment along with systemic therapy would go a long way in controlling the transmission of hanseniasis.—Authors' Abstract

Richardus, J. H. and Smith, T. C. Squamous cell carcinoma in plantar ulcers in leprosy; a case-control study. Lepr. Rev. **64** (1993) 270–274.

The objective of this case-control study was to identify factors associated with the development of squamous cell carcinoma (SCC) in plantar ulcers of leprosy patients. We examined two matched groups consisting of leprosy patients with and without SCC in a plantar ulcer. No correlations were found between the development of SCC and race, profession, place of origin, duration of leprosy, the type and duration of leprosy chemotherapy, presence of bone involvement and type of ulcer care treatment given. The only statistically valid finding was that the duration of the ulcer was significantly lower in the group with malignant change. In this group there was an apparently higher use of pesticides, the difference being not of statistical significance. It is concluded that factors other than ulcer duration need to be looked for, in order to identify factors influencing malignant change in plantar ulcers of leprosy patients.—Authors' Summary

Salafia, A. and Chauhan, G. Gangliosides (cronassial) and nerve regeneration in leprosy; a multicenter clinical trial. Rev. Leprol. Fontilles **19** (1993) 57–72.

Cronassial (a highly purified extract of four active gangliosides) has been used in 226 leprosy patients who had peripheral neuropathies. The present report deals in details with the results achieved in the sensory modalities, with a short note on the motor and sympathetic changes: 15.5% of patients had excellent results, i.e., had complete sensory recovery, while 44% had good results. In 30% of chronic plantar ulcers a significant improvement was noted. Improved sweating function and hair regrowth in a few patients is recorded. The role played by gangliosides in nerve regeneration is discussed briefly.—Authors' Summary

Yan, L., *et al.* [Analysis of 2114 lagophthalmos cases in leprosy.] China Lepr. J. **9** (1993) 6–8. (in Chinese)

Among 14,257 cases of leprosy in 11 counties and cities of Yanzhou and Dongtai, Jiangsu Province, 2114 cases of lagophthalmos were found, including 1214 unilateral and 900 bilateral, accounting for 14.83% of all the cases and 72.92% of leprosy eye diseases. In those with lagophthalmos, paralytic ectropion, exposure keratitis and loss of sight were significantly more than in those without lagophthalmos. Age, leprosy type and the disease duration of the patients are significantly related to lagophthalmos. When lagophthalmos occurs, their disease duration of less than 4 years for PB patients is 58.93% with the majority being unilateral, and the duration of more than 10 years is in 69.95% of MB patients with the majority being bilateral. The interval between occurrences of lagophthalmos on two sides is less than 2 months in 90.88%. Lagophthalmos with mimetic paralysis is in 72.23%; 60% of lagophthalmos occurs unconsciously. The authors emphasize that the majority of exposure keratitis and blindness caused by lagophthalmos is preventable.—Authors' English Abstract

Immuno-Pathology

Adams, L. B., Fukutomi, Y. and Krahenbuhl, J. L. Regulation of murine macrophage effector functions by lipoarabinomannan from mycobacterial strains with different degrees of virulence. Infect. Immun. **61** (1993) 4173–4181.

Lipoarabinomannan (LAM) is the major arabinose- and mannose-containing phosphorylated lipopolysaccharide (LPS) in mycobacterial cell walls. LAM preparations from a virulent strain (Erdman) [LAM(Erdman)] and an attenuated strain (H37Ra) [LAM-(H37Ra)] of *Mycobacterium tuberculosis,* as well as from *M. leprae* (a virulent mycobacterium), were analyzed for their effects on various macrophage effector functions. LAM(H37Ra), like gram-negative LPS, exhibited a dose-dependent ability to induce tumor necrosis factor alpha (TNF-α) production in normal macrophages, and gamma interferon (IFN-γ) priming of the macrophages greatly augmented the levels of TNF-α. However, the effects of LAM(H37Ra) were unaffected by polymyxin B, which totally abrogated the effects of LPS. LAM(Erdman) and LAM from *M. leprae,* on the other hand, induced virtually no TNF-α production. Analysis of macrophage mRNA by reverse transcription-polymerase chain reaction revealed that the levels of TNF-α mRNA induced by the various preparations correlated with the levels of TNF-α protein detected. Interestingly, both LAM(H37Ra) and LAM(Erdman) could block subsequent IFN-γ- and LPS-induced macrophage activation, a previously reported measure of the potent ability of LAM to down-regulate macrophage effector functions. Two lines of evidence suggested, however, that cyclooxygenase products did not play a role in this down-regulation. LAM(H37Ra) and LPS could induce the production of NO2− in both normal and IFN-γ-primed macrophages, whereas LAM(Erdman) could stimulate NO2− production only in primed macrophages. Both LAM(H37Ra) and LAM(Erdman) could substitute for LPS as a triggering signal for IFN-gamma-primed macrophages in a toxoplasma killing assay. The triggering ability of LAM(Erdman), however, was abrogated by an anti-TNF-α antibody, suggesting that sufficient TNF-α production was stimulated by LAM(Erdman) to drive a macrophage function relevant in host resistance. Thus, mycobacterial LAM is a potent regulator of macrophage functions, a fact that may have important consequences in mycobacterial disease.—Authors' Abstract

Arruda, H. O., Cedenho, A. P. and Arruda, L. H. F. Testicular alterations in Hansen's disease. Hansenol. Int. **16** (1991) 35–43.

Fifty testicular specimens and seminal fluid of 10 normal individuals and 24 patients with Hansen's disease were studied. General and early histological alterations, the frequency of young spermatides in tubular lumen and the measure of the diameters of 1450 seminiferous tubules were checked. According to the results, it was not possible to establish a qualifying correlation within histological descriptions and/or a quantifying correlation within morphometry from a specific clinical group with Hansen's disease. Thus, the simultaneous appearance and the variability of the findings seen in the same testis are opposite to the evolving phases described by Grabstald and Swan and to the classification presented by Kumar. The authors suggest that the testicular alterations are a consequence of an autoaggression to the gland.—Authors' Abstract

Baumgart, K. W., Britton, W. J., Mullins, R. J., Basten, A. and Barnetson, R. St.C. Subclinical infection with *Mycobacterium leprae*—a problem for leprosy control strategies. Trans. R. Soc. Trop. Med. Hyg. **87** (1993) 412–415.

Tests for the serodiagnosis of leprosy, including those based on phenolic glycolipid-I (PGL-I), have shown poor specificity for leprosy in studies of endemic communities, despite initially promising results in studies of selected patients. During a 5-year follow-up study of a hyperendemic community in Papua New Guinea, a marked reduction in the numbers of seropositive children and an increase in age of those seropositive followed introduction of multidrug therapy.

This was accompanied by a reduced case detection rate and a shift to paucibacillary disease in new cases, consistent with a reduction in transmission. Only a minority of persistently seropositive persons developed leprosy. These observations suggest that subclinical infection with *Mycobacterium leprae* is common in endemic communities and that PGL-I seropositivity is a marker of subclinical infection, with poor specificity for overt disease. Detection of subclinical infection may confound control strategies which use screening tests to identify asymptomatic highly infectious cases for earlier therapy.—Authors' Abstract

Blair, A. L., Cree, I. A., Beck, J. S., Grange, J. M. and Kardjito, T. Heat-stable opsonins in tuberculosis and leprosy. FEMS Immunol. Med. Microbiol. 7 (1993) 197–204.

We have examined heat-stable opsonins to four species of gamma-irradiated mycobacteria (*Mycobacterium tuberculosis* (H37Rv), *M. avium* (28A), *M. scrofulaceum* and *M. leprae* (cd 103)) in complement-depleted sera collected from Indonesian subjects with tuberculosis (106 patients), leprosy (24 patients) and controls (40 hospital workers and 41 factory workers) indirectly by microtiter plate chemiluminescence (CL) assay and compared the results with antibody levels. The results indicate that there is a wide range of opsonic capacity for mycobacteria in complement-depleted sera. There was poor correlation between the opsonic capacity as measured by CL and the anti-mycobacterial antibody content of sera measured by ELISA, suggesting that anti-mycobacterial antibody has little influence on the uptake of mycobacteria. However, a nonspecific heat-stable opsonin appears to be present in some sera. Conversely, some sera from tuberculosis or leprosy patients suppress the production of reactive oxygen species from normal phagocytes *in vitro* when stimulated with *M. tuberculosis*. The relevance of this inhibition and the presence of heat-stable opsonins to the pathogenesis of tuberculosis have yet to be determined, but it is possible that the presence of opsonins may inhibit dissemination of tubercle bacilli to other organs.—Authors' Abstract

Britton, W. J. Leprosy 1962–1992. 3. Immunology of leprosy. Trans. R. Soc. Trop. Med. Hyg. **87** (1993) 508–514.

The host immune response to *Mycobacterium leprae* is critical for control of the infection but also responsible for the immunopathological damage to skin and nerves. The complex and varied immune responses to the organism are the basis for the clinical spectrum of disease ranging from tuberculoid to lepromatous leprosy. The cellular interactions underlying this spectrum are discussed and the antigenic components of the bacillus briefly reviewed. *M. leprae* has evolved a variety of mechanisms to avoid macrophage bactericidal mechanisms. These result in the persistence of bacilli and the release of cytokines leading to chronic granulomatous inflammation. The immune response to *M. leprae* is dynamic and spontaneous variations in cellular reactivity occur with time leading to type 1 and 2 leprosy reactions. The factors which preset the host immune response to a tuberculoid or lepromatous pattern and which precipitate reactional episodes remain to be elucidated.—Author's Abstract

Butt, K. I., Kawatsu, K., Wang, T., Maeda, Y. and Izumi, S. Immunopathological stain of lipoarabinomannan-B (LAM-B) for diagnosis of leprosy. Jpn. J. Lepr. **62** (1993) 13–20.

We developed an immunopathological staining of LAM-B antigen in Formalin-fixed paraffin-embedded tissues and compared it with PGL-I immunostaining, Fite-Faraco's stain and periodic acid carbol pararosaniline (PACPR) stain. Out of the total 28 leprosy cases, 27 were positive to LAM-B immunostaining while 23 were positive to PGL-I stain. Fite's stain was positive in 21 cases while PACPR stain was positive in 24 cases. In scrofuloderma, LAM-B antigen was observed only in the granuloma while no other positive findings were noted with other stains. Normal skin did not give any positive findings with any of the stains. Other dermatoses showed no positive findings to any of the stains tested. LAM-B staining was observed in the nerve even in the absence of bacilli in leprosy tissues. Presence of LAM-B in the cutaneous

nerves is helpful in discriminating leprosy from other mycobacterioses. Considering the high sensitivity of LAM-B and the predilection of *Mycobacterium leprae* for the nerves, we concluded that LAM-B staining can be a useful new tool in the prompt diagnosis of leprosy, especially in suspected or early cases.—Authors' Summary

Can, Y., *et al.* [Study of the IgA antibody to PGL-I specific to *M. leprae.*] China Lepr. J. **9** (1993) 71–74. (in Chinese)

The level of IgA antibody to PGL-I in the serum was determined with NT-P-BSA/ELISA, including 1156 blood specimens which have been taken from 373 leprosy patients, 81 household contacts, 11 occupational contacts, 144 tuberculosis patients, 120 pregnant women and 427 noncontacts. When the cut-off point of the ELISA was defined as 0.04 (OD value), its specificity was 95.6% and sensitivity was 63.0%, with significant difference between leprosy patients and controls ($p < 0.01$). There was a positive correlation between the levels of IgA and IgM in 246 active leprosy patients ($r > 0.6$, $p < 0.001$) and in controls ($r > 0.5$, $p < 0.001$). It might not be useful to determine IgA in screening subclinical infection with *Mycobacterium leprae,* evaluating the effect of therapy and doing relevant immuno-epidemiological study.—Authors' English Abstract

Cooper, A. M., Dalton, D. K., Stewart, T. A., Griffin, J. P., Russell, D. G. and Orme, I. M. Disseminated tuberculosis in interferon-γ gene-disrupted mice. J. Exp. Med. **178** (1993) 2243–2247.

The expression of protective immunity to *Mycobacterium tuberculosis* in mice is mediated by T lymphocytes that secrete cytokines. These molecules then mediate a variety of roles, including the activation of parasitized host macrophages, and the recruitment of other mononuclear phagocytes to the site of the infection in order to initiate granuloma formation. Among these cytokines, interferon γ (IFN-γ) is believed to play a key role is these events. In confirmation of this hypothesis, we show in this study that mice in which the *IFN-γ* gene has been disrupted were unable to contain or control a normally sublethal dose of *M. tuberculosis,* delivered either intravenously or aerogenically. In such mice, a progressive and widespread tissue destruction and necrosis, associated with very high numbers of acid-fast bacilli, was observed. In contrast, despite the lack of protective immunity, some DTH-like reactivity could still be elicited. These data, therefore, indicate that although IFN-γ may not be needed for DTH expression, it plays a pivotal and essential role in protective cellular immunity to tuberculosis infection.—Authors' Summary

Damle, A. and Mahadevan, P. R. *In vivo* effect of delipidified cell component of *Mycobacterium leprae* in relation to infection with leprosy bacteria in mice. Indian J. Lepr. **65** (1993) 271–282.

The delipidified cell component (DCC) of *Mycobacterium leprae* was used as an immunomodulatory agent in Swiss white mice. The peritoneal macrophages of these mice were activated to produce increased amounts of reactive oxygen intermediates like hydrogen peroxide (H_2O_2) and superoxide. These macrophages also attained the ability to kill *M. leprae in vitro* as shown by several assay systems including the conventional mouse foot pad technique. The increased levels of superoxide seem to be responsible for the killing of *M. leprae* as addition of the enzyme superoxide dismutase, which breaks down O_2^-, resulted in survival of these bacilli inside the macrophages. The increased production of H_2O_2 does not seem to be responsible for killing *M. leprae.* The results indicate that the DCC of *M. leprae* acts as an effective immunomodulator in mice leading to the activation of macrophages with increased production of H_2O_2 and superoxide as well as enabling them to kill *M. leprae* via the action of superoxide anions.—Authors' Abstract

Davodeau, F., Peyrat, M. A., Hallet, M. M., Gaschet, J., Houde, I., Vivien, R., Vie, H. and Bonneville, M. Closs correlation between Daudi and mycobacterial antigen recognition by human-gamma/delta-encoded T cells and expression of V9JPC1-delta/V2DJC-delta-encoded T-cell receptors. J. Immunol. **151** (1993) 1214–1223.

Recent studies have demonstrated that a large fraction of human gamma/delta PBL recognize antigens (Ag) of prokaryotic and eukaryotic origins, respectively, found in hydrosoluble mycobacterial extracts and on the Daudi Burkitt's lymphoma cells. The structural basis of the recognition of these Ag has been presently studied in detail, through analysis of a large panel of thymus- and peripheral blood-derived gamma/delta T-cell clones. Our results suggest that Daudi and mycobacteria-reactive gamma/delta subsets are strictly overlapping and, hence, that gamma/delta T-cell responses against these two Ag are closely related. Daudi cells and mycobacteria were recognized by Vgamma9+Vdelta2+, but not by Vgamma9+Vdelta2−, Vgamma9−Vdelta2+, or Vgamma9−Vdelta2− PBL clones. However, not all Vgamma9+Vdelta2+ clones were reactive and, in particular: 1) the proportion of Ag-reactive lymphocytes was much lower among thymus- than PBL-derived clones (respectively, 24/36 vs 72/73); 2) none of the Vgamma9+Vdelta2+ clones expressing a V9J2C2 gamma chain (N = 4) were reactive to Daudi or mycobacteria, indicating that expression of a disulfide-linked TCR is probably a prerequisite for recognition of these Ag; and 3) among Vgamma9+Vdelta2+ clones bearing disulfide-linked TCR, almost all (50/53) clones expressing a V9JPC1 gamma chain were reactive; whereas a large fraction (6/10) of those expressing a V9J1C1 gamma chain were weakly or nonreactive. Together, these observations suggest that germline residues specific to Vgamma9, Vdelta2, and JgammaP elements directly contribute to recognition of Daudi and mycobacterial Ag. Furthermore, these findings may provide an explanation for coordinate use of these gene elements by a large fraction of gamma/delta PBL, through peripheral selection events mediated by ligands identical or structurally related to the above Ag.—Authors' Abstract

Fleury, R. N. [Difficulties with the use of the Ridley-Jopling classification—a morphological analysis.] Hansenol. Int. **14** (1989) 101–106. (in Portuguese)

There are many difficulties in the use of the Ridley-Jopling classification in daily practice. The author identified the morphologic parameters whose variations permit to distinguish the polar types and borderline groups according to Ridley. If we avoid the inconstant histologic alterations we believe that this distinction depends basically on the following parameters: epithelioid cell, granuloma of epithelioid cells, number of lymphocytes and number of bacilli. A critical analysis is performed of each of these parameters and the author concludes that they are scarce, and that there are great difficulties for the identification and interpretation of their variations for classification purposes. These difficulties are even more important during the reactional episodes.—Author's English Abstract

Flynn, J. L., Chan, J., Triebold, K. J., Dalton, D. K., Stewart, T. A. and Bloom, B. R. An essential role for interferon γ in resistance to *Mycobacterium tuberculosis* infection. J. Exp. Med. **178** (1993) 2249–2254.

Tuberculosis, a major health problem in developing countries, has re-emerged in recent years in many industrialized countries. The increased susceptibility of immunocompromised individuals to tuberculosis, and many experimental studies indicate that T-cell-mediated immunity plays an important role in resistance. The lymphokine interferon γ (IFN-γ) is thought to be a principal mediator of macrophage activation and resistance to intracellular pathogens. Mice have been developed which fail to produce IFN-γ (gko), because of a targeted disruption of the gene for IFN-γ. Upon infection with *Mycobacterium tuberculosis,* although they develop granulomas, gko mice fail to produce reactive nitrogen intermediates and are unable to restrict the growth of the bacilli. In contrast to control mice, gko mice exhibit heightened tissue necrosis and succumb to a rapid and fatal course of tuberculosis that could be delayed, but not prevented, by treatment with exogenous recombinant IFN-γ.—Authors' Summary

Ghei, S. K. Agrewala, J. N., Sengupta, U. and Sudhakar, K. S. Group-specific component (Gc) in erythema nodosum leprosum (ENL). Indian J. Lepr. **65** (1993) 323–325.

The distribution of phenotypes of group-specific component (Gc) was examined in 71 lepromatous leprosy (LL) patients without any history of ENL reaction and 65 LL patients with history of frequent episodes of ENL reaction. The distribution of none of the phenotypes of Gc (Gc 1-1, Gc 2-1, Gc 2-2) was statistically significant among these groups.—Authors' Abstract

Godfrey, H. P. T-Cell fibronectin and mycobacterial adversarial strategy. Int. J. Clin. Lab. Res. **23** (1993) 121–123.

Sensitized T cells synthesize and secrete a biochemically and functionally distinct cellular fibronectin in response to antigenic stimulation. This T-cell fibronectin is associated with initiation of *in vivo* delayed hypersensitivity inflammatory reactions. Mycobacterial secretory proteins of the fibronectin-binding antigen 85 complex can bind and inactivate T-cell fibronectin, and diminish expression of anti-mycobacterial delayed hypersensitivity. Interaction of T-cell fibronectin and antigen 85 provides an additional possible mechanism for tuberculin anergy in patients with clinical tuberculosis.—Author's Abstract

Hasan, R. S., Dockrell, H. M., Jamil, S., Chaing, T. J. and Hussain, R. Antigen-coated latex particles as a model system for probing monocyte responses in leprosy. Infect. Immun. **61** (1993) 3724–3729.

To study responses to *Mycobacterium leprae* antigens, we developed an *in vitro* model system in which latex particles coated with *M. leprae* sonic extract (MLSON) antigen were presented to monocytes. Uptake and oxidative response as measured by superoxide production to these antigens were investigated. Phagocytosis of MLSON-coated particles was greater than that of control particles in monocytes from both leprosy patients and controls from leprosy-endemic areas; uptake of MLSON-coated particles was higher in monocytes from lepromatous leprosy patients than in cells from tuberculoid leprosy patients and controls. In both patients and controls, uptake of latex particles coated with leprosy antigens triggered very little reduction of nitroblue tetrazolium although the cells were capable of mounting a respiratory burst. Antigen-coated latex particles can therefore be used as a tool to investigate monocyte responses to *M. leprae* and individual recombinant antigens.—Authors' Abstract

Inaba, K., Inaba, M., Naito, M. and Steinman, R. M. Dendritic cell progenitors phagocytose particulates, including bacillus Calmette-Guérin organisms, and sensitize mice to mycobacterial antigens *in vivo*. J. Exp. Med. **178** (1993) 479–488.

Dendritic cells, while effective in sensitizing T cells to several different antigens, show little or no phagocytic activity. To the extent that endocytosis is required for antigen processing and presentation, it is not evident how dendritic cells would present particle-associated peptides. Evidence has now been obtained showing that progenitors to dendritic cells can internalize particles, including bacillus Calmette-Guérin (BCG) mycobacteria. The particulates are applied for 20 hr to bone-marrow cultures that have been stimulated with granulocyte/macrophage colony-stimulating factor (GM-CSF) to induce aggregates of growing dendritic cells. Cells within these aggregates are clearly phagocytic. If the developing cultures are exposed to particles, washed, and "chased" for 2 days, the number of major histocompatibility complex class II-rich dendritic cells increases substantially and at least 50% contain internalized mycobacteria or latex particles. The mycobacteria-laden, newly developed dendritic cells are much more potent in presenting antigens to primed T cells than corresponding cultures of mature dendritic cells that are exposed to a pulse of organisms. A similar situation exists when the BCG-charged dendritic cells are injected into the foot pad or blood stream of naive mice. Those dendritic cells that have phagocytosed organisms induce the strongest T-cell responses to mycobacterial antigens in draining lymph node and spleen. The administration of antigens to GM-CSF-induced, developing dendritic cells (by increasing both antigen uptake and cell numbers) will facilitate the use of these antigen-presenting cells for active immunization *in situ*.—Authors' Abstract

Kar, H. K., Sharma, A. K., Misra, R. S., Beena, K. R., Zaheer, S. A., Mukherjee, R., Mukherjee, A., Parida, S. K., Walia, R., Nair, N. S. K. and Talwar, G. P. Reversal reaction in multibacillary leprosy patients following MDT with and without immunotherapy with a candidate for an antileprosy vaccine, *Mycobacterium w.* Lepr. Rev. **64** (1993) 219–226.

Immunotherapy with a candidate for an antileprosy vaccine, *Mycobacterium w,* was given in addition to standard multidrug therapy (MDT) to 53 multibacillary lepromin-negative patients belonging to BB, BL and LL types of leprosy (vaccine group). An equal control group received MDT and injections of micronized starch as placebo. Both the vaccine and placebo were administered intradermally every 3 months. The patients were evaluated at determined intervals by clinical, bacteriological and histopathological parameters and lepromin testing. Reactional episodes were analyzed with reference to incidence, onset, frequency and severity during and after release from treatment (RFT). Incidence of reversal reaction (RR) was marginally higher in the vaccine group (22.6% vaccine group vs 15% control group). All cases with a history of downgrading type 1 reaction developed RR during therapy. Most episodes occurred within the first year of the commencement of therapy—50% developing within 3 months. Late reversal reactions (after RFT) were observed in 3.8% of cases in both groups, and 50% of the reactors in the control group and 33% in the vaccine group had repeated reactional episodes. Incidence of neuritis associated with RR as well as isolated neuritis was similar in both groups.—Authors' Summary

Khare, S., Bhutani, L. K. and Rao, D. N. Modulation of peripheral blood derived monocytes/macrophages from leprosy patients using "tuftsin" for production of reactive oxygen intermediates. Lepr. Rev. **64** (1993) 208–218.

Phagocytic cells respond to a variety of membrane stimulants by producing reactive oxygen intermediates (ROI), i.e., O_2^-, H_2O_2 and OH· metabolites. Plasma membrane activation is associated with superoxide generating NADPH oxidase, thereby causing the production of these toxic species. Stimulation of phagocytic cells also results in activation of purine catabolism, which directs the metabolic flux through xanthine oxidase to produce the superoxide anion. We previously observed that BL/LL macrophages (Mϕ) exhibited a premature inability to undergo tuftsin-stimulated phagocytosis and microbicidal activity. The present study was undertaken to measure ROI levels in the absence and presence of "tuftsin" pulsing as a function of *in vitro* culture age and also correlated these levels with adenosine deaminase (ADA) activity. The latter is known to be a contributor of O_2^- generation and is also involved in the maturation of the monocyte/macrophage system. The behavior of normal and tuberculoid monocytes/macrophages was more or less the same, either in the presence or absence of tuftsin, i.e., they showed a progressive increase in ROI production until day 3, then tapered off in older cultures by day 7. In contrast, after day 1, the lepromatous macrophages were unable to undergo tuftsin-mediated stimulation for the production of ROI and ADA activity. These findings indicate a defective Mϕ function in lepromatous patients toward tuftsin pulsing, thereby supporting our earlier observations. Thus BL/LL Mϕ behaved as if they were aged after 1 day of *in vitro* culture, which may account for an inability to handle *Mycobacterium leprae* for efficient killing.—Authors' Summary

Kroumpouzos, G., Vareltzidis, A., Konstadoulakis, M. M., Avgerinou, G., Anastasiadis, G., Kroubouzou, H., Panteleos, A. and Tosca, A. Evaluation of the autoimmune response in leprosy. Lepr. Rev. **64** (1993) 199–207.

Immunological responses to a panel of antigens were evaluated in 27 patients with lepromatous and 20 patients with tuberculoid leprosy and compared with 24 pulmonary tuberculosis patients, 25 systemic lupus erythematosus patients and 41 healthy blood donors. Some autoantibody specificities were extensively studied for the first time in mycobacterial infections. Striking immunoserological abnormalities were found in patients with lepromatous leprosy, particularly in those presenting with re-

lapse. Inhibition assays were performed providing a tool for further analysis of the binding range of specific anti-ND-O-BSA antibodies and strengthening the suggestion of molecular mimicry reactions between cytoskeletal proteins, host stress proteins and *Mycobacterium leprae* antigens or stress proteins. A significant serological overlap between lepromatous leprosy and autoimmune diseases is indicated.—Authors' Summary

Locksley, R. M., Reiner, S. L., Hatam, F., Littman, D. R. and Killeen, N. Helper T cells without CD4: control of leishmaniasis in CD4-deficient mice. Science **261** (1993) 1448–1451.

Expression of either the CD4 or CD8 glycoproteins discriminates two functionally distinct lineages of T lymphocytes. A null mutation in the gene encoding CD4 impairs the development of the helper-cell lineage that is normally defined by CD4 expression. Infection of CD4-null mice with *Leishmania* has revealed a population of functional helper-T cells that develops despite the absence of CD4. These $CD8^-$ $\alpha\beta$T cell receptor$^+$ T cells are major histocompatibility complex class II-restricted and produce interferon-γ when challenged with parasite antigens. These results indicate that T-lymphocyte lineage commitment and peripheral function need not depend on the function of CD4.—Authors' Abstract

Makonkawkeyoon, S., Limson-Pobre, R. N. R., Moreira, A. L., Schauf, V. and Kaplan, G. Thalidomide inhibits the replication of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. U.S.A. **90** (1993) 5974–5978.

Thalidomide, a selective inhibitor of tumor necrosis factor α (TNF-α) synthesis, suppresses the activation of latent human immunodeficiency virus type 1 (HIV-1) in a monocytoid (U1) line. The inhibition is dose dependent and occurs after exposure of the cells to recombinant TNF-α, phorbol myristate acetate, lipopolysaccharide, and other cytokine combinations. Associated with HIV-1 inhibition is a reduction in agonist-induced TNF-α protein and mRNA production. Thalidomide inhibition of virus replication in the phorbol myristate acetate- and recombinant TNF-α-stimulated T-cell line ACH-2 is not observed. The presence of thalidomide also inhibits the activation of virus in the peripheral blood mononuclear cells of 16 out of 17 patients with advanced HIV-1 infection and AIDS. These results suggest the use of thalidomide in a clinical setting to inhibit both virus replication and the TNF-α-induced systemic toxicity of HIV-1 and opportunistic infections.—Authors' Abstract

Meng, M., *et al.* [On criteria for the positivity in determination of antibody against PGL-I with ELISA.] China Lepr. J. **9** (1993) 13–18. (in Chinese)

Sera from 969 healthy subjects and 974 leprosy patients were tested for anti-PGL-I antibody (APGL-I) by the ELISA technique with NT-P-BSA. The results showed that 1) the mean OD and positivity of healthy females were significantly higher than those of healthy males and a similar tendency occurred between sex groups of leprosy patients, suggesting that the female-positive criteria of OD must be separate from that in the male; 2) The levels of PGL-I were skewed in distribution in all groups except the MB patient group, suggesting it unsuited to calculating the cut-off point of OD according to mean OD + 2 S.D. based on normal distribution; 3) When the leprosy prevalence was reduced to a certain extent, this detection could not reflect slight differences, but might reflect epidemiological dynamics in some epidemic points with relatively higher incidence; 4) There were still significantly higher mean OD and positivity in inactive MB and PB patients than in healthy subjects. It remains to be shown what is clinically and epidemiologically significant and if they could serve as an applicable parameter for relapse; 5) In order to guarantee reliability of detection, it must be emphasized that standardized methods and materials, especially the control pool sera, are necessary for correct results, at different times and in different laboratories.—Authors' English Abstract

Mustafa, A. S. and Oftung, F. Long-lasting T-cell reactivity to *Mycobacterium leprae* antigens in human volunteers vaccinated with killed *M. leprae*. Vaccine **11** (1993) 1108–1118.

A trial with a candidate antileprosy vaccine based on killed *Mycobacterium leprae* was started in Norway in 1983 to evaluate its toxicity and efficacy to induce cell-mediated immunity (CMI) in BCG-vaccinated healthy volunteers. The vaccinated subjects were found to be free of unacceptable side effects and their T cells showed elevated proliferative response to *M. leprae* up to 1 year postvaccination. When tested in 1991, 8 years after vaccination, peripheral blood mononuclear cells from the same volunteers showed a persistent high proliferative response to *M. leprae*. From a total of 147 T-cell clones established from these subjects, 26 clones were specific to *M. leprae* and the remaining T-cell clones responded to *M. leprae* as well as to BCG and other cultivable mycobacteria. The epitopes recognized by the *M. leprae*-specific T-cell clones were present on several protein antigens including the 18-kDa and the 65-kDa heat-shock proteins. A dominant epitope, peptides 38–50 on the *M. leprae* 18-kDa heat-shock protein, which was recognized by *M. leprae*-specific T cells 1 year after vaccination, was also recognized 8 years after vaccination by the same donor. This is the first report demonstrating the unique property of killed *M. leprae* with respect to the induction of long-lasting T-cell reactivity toward *M. leprae* antigens in humans.—Authors' Abstract

Mutis, T., Cornelisse, Y. E. and Ottenhoff, T. H. M. Mycobacteria induce CD4+ T cells that are cytotoxic and display Th1-like cytokine secretion profile—heterogeneity in cytotoxic activity and cytokine secretion levels. Eur. J. Immunol. **23** (1993) 2189–2195.

Protective immunity against mycobacteria is dependent on antigen-specific T cells. Current evidence suggests that not only helper-T cells that activate infected macrophages but also cytotoxic-T cells (CTL) that lyse infected macrophages are involved in protection. Mycobacterium-specific CD4+ CTL are readily detectable among primary peripheral T cells but what proportion of CD4+ T cells display cytotoxic activity is not known. Whether the cytotoxic CD4+ T cells are identical to or distinct from those that produce interferon (IFN)-gamma is also unknown. In addition, studies on CTL in mycobacterial infections have focused primarily on selected antigens like hsp65 but have not analyzed systematically whether other mycobacterial antigens can activate CTL as well. These issues are relevant not only to a further understanding of protective immunity and immunopathology but also may have implications for the design of effective vaccines. To start addressing these issues, we have studied a large panel of CD4+ T-cell clones specific for a broad range of mycobacterial antigens, and analyzed their ability to lyse mycobacterium-pulsed target cells and to release IFN-gamma and interleukin (IL)-4. Our results show that the vast majority of CD4+ T-cell clones are able to lyse mycobacterial antigen-pulsed target cells, and that those CTL can be triggered by a wide variety of mycobacterial antigens. CD4+ CTL released high levels of IFN-gamma, but low or nondetectable levels of IL-4. In contrast, control tetanus toxoid-specific T-cell clones or lines displayed poor or weak cytotoxic activity and released high levels of IL-4. The antimycobacterial clones appeared to be heterogeneous in their levels of cytotoxic activity and IFN-gamma release. Interestingly one T-cell clone was able to lyse only mycobacterium-pulsed macrophages but not B cells, suggesting possible selectivity in target cell recognition for some CTL. These *in vitro* data have to be interpreted with some caution. Nevertheless they confirm and significantly extend previous observations, and suggest that mycobacteria preferentially induce CD4+ T-helper type 1 (Th1)-like cells that display cytotoxic activity, and release high levels of IFN-gamma but no or little IL-4. The induction of such Th1-like cells is specific for mycobacteria since tetanus toxoid induced T cells that were poorly or not cytolytic and secreted high levels of IL-4.—Authors' Abstract

Nibbering, P. H., Pos, O., Stevenhagen, A. and Van Furth, R. Interleukin-8 enhances nonoxidative intracellular killing of *Mycobacterium fortuitum* by human granulocytes. Infect. Immun. **61** (1993) 3111–3116.

The results of this study show that recombinant interleukin-8 (IL-8) enhances the intracellular killing of *Mycobacterium fortuitum* by human granulocytes. This chemokine did not stimulate the phagocytosis of *M. fortuitum* by granulocytes at various bacterium-to-cell ratios. The killing process was not affected by the NADPH oxidase inhibitor diphenyleneiodonium bisulfate, which indicates that recombinant IL-8 stimulates oxygen-independent mycobactericidal mechanisms of granulocytes. IL-8 did not stimulate H_2O_2 production in granulocytes but primed the cells for enhanced H_2O_2 production upon stimulation with preopsonized *M. fortuitum*. In sum, the chemokine IL-8 not only is involved in the recruitment of granulocytes to the site of infection but also facilitates the elimination of microorganisms by increasing the efficiency of the bactericidal activity of granulocytes.—Authors' Abstract

Pearlman, E., Kazura, J. W., Hazlett, R. E. and Boom, W. H. Modulation of murine cytokine responses to mycobacterial antigens by helminth-induced T-helper-2 cell responses. J. Immunol. **151** (1993) 4857–4864.

BALB/c mice inoculated with live *Brugia malayi* microfilariae (mf), or immunized with a soluble filarial extract (*B. malayi* Ag (BmA)), develop a pronounced Th2-like response over time. In contrast, single or repeated immunizations with a soluble *Mycobacterium tuberculosis* Ag preparation (purified protein derivative, PPD) stimulates a Th1, but not Th2 response (IFN-gamma ≫ IL-4, IL-5). To determine if the Th1 response to PPD can be modulated by the ongoing helminth-induced Th2 activity, mice were: 1) immunized simultaneously with BmA and PPD; 2) immunized first with BmA, then with PPD; or 3) inoculated with live mf and immunized with PPD at various times thereafter. Simultaneous immunization with both Ag had no effect on the Th response induced by PPD, i.e., it was strictly Th1. In contrast, establishment of a Th2 response by either inoculation of live mf or immunization with BmA before administration of PPD skewed the PPD-specific Th response such that IL-4 and IL-5 were produced in addition to IFN-gamma. IL-4 and IL-5 levels produced in response to PPD under these conditions were further elevated *in vitro* in the presence of neutralizing IFN-gamma. Finally, *in vivo* neutralization of IL-4 diminished induction of Th2 responses to PPD. These results demonstrate that ongoing Th2 responses to helminth Ag modulate the Th response to mycobacterial Ag by an IL-4 dependent mechanism.—Authors' Abstract

Rani, R., Fernandez Vina, M. A., Zaheer, S. A., Been, K. R. and Stastny, P. Study of HLA class-II alleles by PCR oligotyping in leprosy patients from north India. Tissue Antigens **42** (1993) 133–137.

Host factors seem to play an important role in determining the immune response and the differential manifestations of lepromatous (LL) and tuberculoid (TT) leprosy. In order to investigate the role of immunogenetic factors in determining the form of leprosy, the HLA class II alleles of DRB1, DRB3, DRB5, DQA1, DQB1 and DPB1 were studied by a PCR oligotyping technique in 93 patients and 47 healthy controls. DRB1*1501 and DRB1*1502 (two of five tested subsets of the serologically defined DR2) accounted for 81.5% of the multibacillary patients (relative risk 16.3) and 60.7% of the TT patients (relative risk 5.7) compared to 21.3% in normal, ethnically and geographically matched controls. The much stronger association of DRB1*1501 with the multibacillary form than with the TT type of leprosy suggests a possible role in the differential immune response to *Mycobacterium leprae* antigens. DQB1*0601 was found significantly more often than in controls throughout the leprosy spectrum, while DQA1*0103 was most frequent in the LL group and DQA1*0102 was selectively increased in the borderline lepromatous (BL) patients. On the other hand, DRB1*0701, DQB1*0201 and DQA1*0201 were decreased in the multibacillary leprosy patients compared to TT patients and controls, and DQB1*0503 was selectively decreased in TT patients, suggesting that these HLA alleles might play a role in modulating the immune response that determines the form of leprosy that develops in each patient.—Authors' Abstract

Sampaio, E. P., Kaplan, G., Miranda, A., Nery, J. A. C., Miguel, C. P., Viana, S. M. and Sarno, E. N. The influence of thalidomide on the clinical and immunologic manifestation of erythema nodosum leprosum. J. Infect. Dis. **168** (1993) 408–414.

Immunologic and clinical manifestations of erythema nodosum leprosum (ENL) and their response to thalidomide therapy were evaluated. Circulating tumor necrosis factor-alpha (TNFα) levels were assayed in serum obtained from lepromatous leprosy patients at diagnosis, during multidrug therapy, at the onset of ENL episodes, and during treatment with thalidomide. Patients with systemic ENL demonstrated the highest serum TNF-α levels, which decreased significantly during thalidomide treatment. Serum TNF-α in nonreactional patients was associated with mild flu-like symptoms and local inflammatory lesions. Serum interferon-gamma (IFN-γ) was also elevated in patients with high TNF-α levels. Thalidomide therapy reduced not only serum TNF-α levels and the clinical symptoms but also the dermal infiltration of polymorphonuclear leukocytes and T cells. The expression of intercellular adhesion molecule 1 and major histocompatibility complex class II antigens on the epidermal keratinocytes was also downregulated. These results indicate that the thalidomide-induced alleviation of clinical symptoms of ENL was associated with a reduction of TNF-α levels.—Authors' Abstract

Santos, D. O., Suffys, P. N., Bonifacio, K., Marques, M. A. and Sarno, E. N. *In vitro* tumor necrosis factor production by mononuclear cells from lepromatous leprosy patients and from patients with erythema nodosum leprosum. Clin. Immunol. Immunopathol. **67** (1993) 199–203.

The production of tumor necrosis factor (TNF) by *Mycobacterium leprae*-stimulated phagocyte cells, isolated from lepromatous leprosy patients (LL) and normal individuals, was evaluated, using the highly TNF-sensitive mouse fibrosarcoma cell line WEHI164cl13. Mononuclear cells, isolated from all individuals studied, showed a low level of spontaneous TNF production, except for patients undergoing erythema nodosum leprosum (ENL), in whom we found significantly higher levels of TNF. Addition of *M. leprae* to the phagocyte cell culture enhanced TNF production in all groups studied, except in the group with untreated leprosy patients. Strongest *M. leprae*-induced TNF release was found in mononuclear cell cultures derived from ENL patients. Patients in the postreactional state showed significantly higher TNF levels than healthy controls. These findings support the idea that TNF plays a key role in the complex symptomatology of ENL.—Authors' Abstract

Santos, E. M. C. and Fleury, R. N. [Histopathologic correlations between skin and lymph nodes in the phases of evolution of virchovian hanseniasis.] Hansenol. Int. **15** (1990) 58–66. (in Portuguese)

An analysis of evolutive correlation in skin and lymph nodes of 30 patients with lepromatous leprosy was performed. Ten were active lepromatous in progression, 10 active lepromatous in regression and 10 residual lepromatous (inactive). The analysis of the skin and lymph node biopsies in the two former groups showed a remarkable heterogeneity in the constitution of specific granuloma in lymph nodes contrasting with the homogeneous constitution of cutaneous granuloma. In the third group whose material was taken from necropsies, the residual infiltrates in skin were discrete, meanwhile the lymph nodes maintained, in the majority of cases, extensive paracortical, residual, macrophagic granulomas. The macrophagic granuloma in lymph nodes ordinarily showed a characteristic stratification with a tendency of younger macrophages and solid bacilli to locate in cortical and paracortical areas; whereas macrophages with regressive characteristics containing granular bacilli were situated in deep paracortical areas. There was an intense depletion of paracortical lymphocytic population, follicular hyperplasia and numerous plasma cells in the active lepromatous; whereas in residual lepromatous the follicular hyperplasia disappears, medular plasma cells are maintained and the paracortical lymphocytic depletion remains. Three regressive and one residual lepromatous pre-

sented alterations peculiar to erythema nodosum leprosum in lymph nodes and perivascular amyloid deposition were found in two inactive lepromatous cases.—Authors' English Abstract

Trindade, M. A. B., Fleury, R. N. and Petri, V. [Clinical and histologic evaluation of the Mitsuda reaction in consanguineous and non-consanguineous contacts of patients with bacilliferous forms of hanseniasis.] Hansenol. Int. **16** (1991) 16–22. (in Portuguese)

Mitsuda tests, clinically and histologically evaluated, were performed on 17 close consanguineous relatives and 23 non-consanguineous healthy contacts of patients exhibiting the bacilliferous forms of leprosy. The proportion of histologically negative Mitsuda reactions among the consanguineous contacts (35.3%) was significantly larger than that observed among the non-consanguineous contacts (8.7%). Clinically positive (3) and doubtful (1) Mitsuda reactions without histological correspondence were found among the consanguineous but not among the non-consanguineous contacts of leprosy cases. The present results indicate that a positive Mitsuda reaction shall only be ascribed to consanguineous contacts of patients with bacilliferous forms of leprosy if confirmed by histological analysis.—Authors' English Abstract

Yadava, A. and Mukherjee, R. An immunodominant 30-kDa antigen of a candidate antileprosy vaccine, *Mycobacterium w*, shares T- and B-cell determinants with *M. leprae* and *M. tuberculosis.* Med. Microbiol. Immunol. **182** (1993) 243–253.

Earlier we reported that vaccination of leprosy patients with *Mycobacterium w* induces an immune response directed predominantly against low-molecular-weight antigens. One of these antigens, with a molecular mass of 30-kDa, was recognized by a majority of the vaccinated subjects as well as the tuberculoid leprosy patients and healthy contacts. In the present communication we report further characterization of this antigen. Immunofluorescence and Western blot studies with antibodies raised against this antigen demonstrate that it is associated with the cell surface and has homologs present in *M. leprae* and *M. tuberculosis.* Delayed-time hypersensitivity studies carried out in guinea pigs immunized with the 30-kDa antigen show that in addition to sharing B-cell determinants, this immunodominant antigen of *Mycobacterium w* also shares T-cell determinants with *M. leprae* and *M. tuberculosis.*—Authors' Abstract

Zhang, X. and Wang, D. [Logistic regression analysis of humoral immune factors in ENL cases.] China Lepr. J. **9** (1993) 74–77. (in Chinese)

On the basis of the data in the determination of the humoral immune factors in 88 cases of leprosy, multifactorial analysis has been done by using logistic regression for finding the correlation probably existing between some immunological factors and ENL. The results showed that the occurrence of ENL is influenced by many factors, of which the most important are of six kinds, that is, CIC, C_3, CH50, antibody to PGL-I, IgM and IgG. The authors put forward a preliminary hypothesis on the pathogenesis of ENL on the basis of the analysis.—Authors' English Abstract

Zhang, Z. and Huang, L. [Contents of superoxide dismutase and lipid-peroxide in the blood of leprosy patients.] China Lepr. J. **9** (1993) 78–80. (in Chinese)

The superoxide anion ($\cdot O_2^-$) is an important bactericide of phagocytes. The concentration of $\cdot O_2^-$ in the body is directly affected by superoxide dismutase (SOD). To clarify the relationship between the metabolic disorder of free radical in leprosy patients and the pathogenesis of leprosy, the content changes of SOD and lipid-peroxide (LPO) in the body of persons with leprosy (LL, BL, TT and BT) and common dermatoses and healthy controls were measured. The results showed that in active MB leprosy patients the content of SOD is higher than that in the MB cures, PB leprosy patients, persons with dermatoses and controls ($p < 0.05$), but the content of LPO is lower than that in the persons with dermatoses and controls ($p < 0.01$). It is suggested that the increased concentration of SOD in the blood of MB leprosy patients might be related to pathogenesis of leprosy.—Authors' English Abstract

Microbiology

Aldovini, A., Husson, R. N. and Young, R. A. The *uraA* locus and homologous recombination in *Mycobacterium bovis* BCG. J. Bacteriol. **175** (1993) 7282–7289.

Molecular genetic manipulation of mycobacteria would benefit from the isolation of mycobacterial genes that could serve both as genetic markers and as sequences used to target homologous integration of recombinant DNA into the genome. We isolated the *Mycobacterium bovis* BCG gene encoding orotidine-5′-monophosphate decarboxylase (OMP-DCase) by complementing an *Escherichia coli* mutant defective in this activity. The BCG OMP-DCase gene (*uraA*) and the flanking DNA were sequenced. The predicted BCG OMP-DCase protein sequence is closely related to the *Myxococcus xanthus* OMP-DCase and more distantly related to the other known prokaryotic and eukaryotic OMP-DCases. To investigate whether homologous integration can occur in *M. bovis* BCG, an improved protocol for transformation of BCG was developed and a linear fragment of mycobacterial DNA containing the *uraA* locus, marked with a kanamycin resistance gene, was introduced into BCG cells by electroporation. The kanamycin-resistant BCG transformants all contained vector DNA integrated into the genome. The marked DNA had integrated into the homologous *uraA* locus in approximately 20% of the transformants. These results have implications for understanding the role of mycobacterial genes in disease pathogenesis and for the genetic engineering of improved mycobacterial vaccines.—Authors' Abstract

Andrew, P. W. and Roberts, I. S. Construction of a bioluminescent mycobacterium and its use for assay of antimycobacterial agents. J. Clin. Microbiol. **31** (1993) 2251–2254.

To show, as a model system, that mycobacteria can express heterologous luciferase genes and that bioluminescence can be a rapid method of measuring antimycobacterial activity, a bioluminescent form of *Mycobacterium smegmatis* was made by transformation with a *Mycobacterium-Escherichia coli* shuttle vector containing the *luxAB* genes from *Vibrio harveyi*. The antimycobacterial effects of antibiotics and biocides could be assayed in real time by using bioluminescent *M. smegmatis*.—Authors' Abstract

Chaicumpar, K., Kohsaka, K., Matsuoka, M. and Nomaguchi, H. Construction of genomic DNA library of *Mycobacterium leprae* Thai-53 strain and detection of *M. leprae*-specific genes in the library by polymerase chain reaction. Jpn. J. Lepr. **62** (1993) 28–32.

A *Microbacterium leprae* genomic library was constructed in the expression vector lambda gt11 by using the DNA derived from *M. leprae* strain Thai 53 which has been passaged in nude mice. This library contained *M. leprae* DNA. The presentation of specific *M. leprae* DNA as the inserts in the vector was shown by polymerase chain reaction using the sets of primers which were specific for the repetitive sequence gene, 36-kDa sequence gene of *M. leprae* and lambda gt11.—From the Article

Clavel-Seres, S., Houssaini Iraqui, M. and Rastogi, N. Extraction and preliminary characterization of a mycobacteriolytic preparation (stazyme) from a staphylococcus strain—mycobacteriocidal activity and its use in rapid extraction of mycobacterial DNA. Curr. Microbiol. **27** (1993) 289–293.

The clear culture filtrate from 1 L culture of a laboratory contaminant of *Staphylococcus* (coagulase-strain, designated *clavelis*) was filtered, concentrated, dialyzed, and the proteins were precipitated. The precipitate was washed, concentrated, and aliquoted (about 4 mg of total proteins/ml, designated as "stazyme"). The crude preparation was subjected to gel filtration on Sephadex G-75, and the fractions were screened for their lytic ability against *Mycobacterium smegmatis*. Native proteins in the "stazyme" were electrophoretically separated, electroeluted, and their lytic activity against *M. smegmatis* was compared with parallel controls (partially purified proteins

extracted from the same quantity of the uninoculated bacterial growth medium). Only "stazyme" preparations caused significant growth inhibition of *M. smegmatis, M. chelonae, M. xenopi, M. tuberculosis,* and *M. kansasii.* "Stazyme" essentially possessed a lytic activity measured with purified *M. smegmatis* and *Micrococcus lysodeikticus* cell walls and showed high bactericidal activity against *M. smegmatis, M. chelonae,* and *M. tuberculosis.* It was also able to rapidly lyse intact *M. smegmatis* organisms, permitting significant yield of mycobacterial DNA.—Authors' Abstract

Coene, M., De Kesel, M., Hong, N. T. T., Gheysen, A., Jezierskaanczukow, A. and Cocito, C. Comparative analysis of the genomes of mycobacteriophages infecting saprophytic and pathogenic mycobacteria. Arch. Virol. **133** (1993) 39–49.

The genomes of a series of mycobacteriophages have been analyzed to disclose possible relationships between genetic characteristics and host range. The percent guanine-plus-cytosine in the DNA of 14 phages was found to be 34.4 to 47.5, as determined by a double-labeling procedure, which is unaffected by the presence of modified bases. The DNA of few mycobacteriophages yielded discordant values when the G + C content was estimated by buoyant density determination and by the double labeling procedure. This observation suggests the possible presence of modified bases in these genomes. The reduced susceptibility of viral DNAs to several restriction endonucleases is suggestive of the occurrence of both methyladenine and methylcytosine in the genome of all the mycobacteriophages studied. Heterologous annealing among the 14 DNAs analyzed yielded 6 hybridization groups. Within one group, the homology level among viral genomes was estimated by comparing the electrophoretic mobilities of restriction fragments: values of 0.8% to 1.3% base substitution have thus been found. A comparison of the genomic characteristics and host range of the mycobacteriophages analyzed suggests a possible relationship between restriction pattern, G + C content, crosshybridization level and host range.—Authors' Abstract

Colston, M. J. Leprosy 1962–1992. 2. The microbiology of *Mycobacterium leprae*; progress in the last 30 years. Trans. R. Soc. Trop. Med. Hyg. **87** (1993) 504–507.

Over the last 30 years, there have been dramatic changes in the way *Mycobacterium leprae* is studied in the microbiology laboratory. The organism still has not been grown *in vitro* but, starting with demonstration of growth in the foot pads of mice and culminating in the application of molecular biological and genetic techniques, we are now in a position to circumvent some of the difficulties arising from lack of cultivability. Such studies are providing us with new insights into the basic biology of the organism and are likely to provide new tools which will be of value in the clinical laboratory. In this article, I briefly outline the progress which has been made, and the potential applications of molecular techniques in such areas as bacterial identification and drug-resistance testing.—Author's Abstract

Daffe, M., McNeil, M. and Brennan, P. J. Major structural features of the cell wall arabinogalactans of *Mycobacterium, Rhodococcus,* and *Nocardia* spp. Carbohydr. Res. **249** (1993) 383–398.

The cell wall arabinogalactans of strains of *Mycobacterium, Rhodococcus,* and *Nocardia* were per-*O*-methylated, partially hydrolyzed with acid, and the resulting oligosaccharides were reduced and per-*O*-ethylated to yield per-*O*-alkylated oligoglycosyl alditol fragments. Analyses of these fragments by gas chromatography-mass spectrometry and of the intact solubilized polysaccharides by H-1 and C-13 NMR revealed the major structural features of the different arabinogalactans from representatives of the different genera. All of the mycobacterial products contained a homogalactan segment of alternating 5-linked alpha-galactofuranosyl (Galf) and 6-linked beta-Galf residues. The arabinan segment consisted of three major domains, linear 5-linked alpha-arabinofuranosyl (Araf) residues and branched (3 → 5)-linked Araf units substituted with either 5-linked Araf or the disaccharide beta-Araf-(1 → 2)-alpha-Araf at both branched positions. The recognition of these features in *in vivo*-grown *Mycobac-*

terium leprae is an important development. The arabinan from strains of *Nocardia* contains a nonreducing-end motif composed of the linear trisaccharide, beta-Araf-(1 → 2)-alpha-Araf-(1 → 5)-Araf, attached to linear 5-linked alpha-Araf units. The galactan segment of the arabinogalactan of *Nocardia* sp. is composed of linear 5-linked beta-Galf units substituted in part at O-6 with terminal beta-glucosyl units. The two representative strains of *Rhodococcus* also differed in the composition of the galactan moiety; in addition to the 5-linked Galf, 2- and 3-linked beta-Galf units are present. The reducing end of the galactans, and therefore, apparently, of the entire arabinogalactans from all species from all genera, are apparently composed of the unit, rhamnosyl-(1 → 3)-N-acetyl-glucosamine, which, in turn, is apparently attached to peptidoglycan via phosphodiester linkage.—Authors' Abstract

Das Gupta, S. K., Bashyam, M. D. and Tyagi, A. K. Cloning and assessment of mycobacterial promoters by using a plasmid shuttle vector. J. Bacteriol. **175** (1993) 5186–5192.

We have constructed a promoter selection vector for mycobacteria to analyze the sequences involved in mycobacterial transcriptional regulation. The vector pSD7 contains extrachromosomal origins of replication from *Escherichia coli* as well as from *Mycobacterium fortuitum* and a kanamycin-resistance gene for positive selection in mycobacteria. The promoterless chloramphenicol acetyltransferase (CAT) reporter gene has been used to detect mycobacterial promoter elements in a homologous environment and to quantify their relative strengths. Using pSD7, we have isolated 125 promoter clones from the slowly growing pathogen *M. tuberculosis* H37Rv and 350 clones from the fast-growing saprophyte *M. smegmatis*. The promoters exhibited a wide range of strengths, as indicated by their corresponding CAT reporter activities (5 to 2500 nmol/min/mg of protein). However, while most of the *M. smegmatis* promoters supported relatively higher CAT activities ranging from 100 to 2500 nmol/min/mg of protein, a majority of those from *M. tuberculosis* supported CAT activities ranging from 5 to only about 100 nmol/min/mg of protein. Our results indicate that stronger promoters occur less frequently in the case of *M. tuberculosis* compared with *M. smegmatis*. To assess the extent of divergence of mycobacterial promoters vis-à-vis those of *E. coli*, the CAT activities supported by the promoters in *E. coli* were measured and compared with their corresponding activities in mycobacteria. Most of the mycobacterial promoter elements functioned poorly in *E. coli*. The homologous selection system that we have developed has thus enabled the identification of mycobacterial promoters that apparently function optimally only in a native environment.—Authors' Abstract

Dayanghirang, J. A. and Matsuoka, M. Quantitative determination of PCR products by colorimetric method. Jpn. J. Lepr. **62** (1993) 21–27.

In this study, a new method was tried to estimate PCR products of *Mycobacterium leprae* quantitatively by hybridization with nonisotope probe in a microplate. Target DNA products were adsorbed onto the well and hybridized with probe. The probes labeled with biotin or digoxigenin were prepared by PCR. Their sensitivities were compared. Digoxigenin was ten times higher in sensitivity than biotin at least. No crossreactivity between other PCR products from *M. leprae* DNA and primers has been observed.

Reliability was evaluated by comparing the estimated quantity of PCR products between this method and the ethidium-bromide-stained agarose gel method. Amplified products from different bacillary numbers of *M. leprae* were estimated by agarose gel electrophoresis. To estimate the concentration of amplified DNA based on intensity of color development, the products were hybridized with a digoxigenin-labeled probe together with DNA of known concentration. The probes were detected by ELISA and OD values were plotted by the extrapolation method. Both agarose gel staining and hybridization procedures yielded results proportional to the original bacillary number. The hybridization result was ten thousand times more sensitive than that of the agarose gel staining method. The

sensitivity of this method allows detection of PCR products at the picogram level.

The plate hybridization method with the digoxigenin-labeled probe offers numerous advantages: shorter washing period, safer than the isotope probe and more objective than agarose gel electrophoresis, aside from its proven high degree of specificity and sensitivity. The potential application of this method for routine quantitative estimation of PCR products was indicated. It may also be said that the original amount of template DNA corresponding to the PCR product is estimated by this method.—Authors' Abstract

Dewit, T. F. R., Bekelie, S., Osland, A., Wieles, B., Janson, A. A. M. and Thole, J. E. R. The *Mycobacterium leprae* antigen-85 complex gene family—identification of the genes for the 85A, 85C, and related MPT51 proteins. Infect. Immun. **61** (1993) 3642–3647.

The genes for two novel members (designated 85A and 85C) of the *Mycobacterium leprae* antigen 85 complex family of proteins and the gene for the closely related *M. leprae* MPT51 protein were isolated. The complete DNA sequence of the *M. leprae* 85C gene and partial sequences of the 85A and MPT51 genes are presented. As in *M. tuberculosis,* the *M. leprae* 85A, 85C, and previously identified 85B component genes are not closely linked on the genome. However, the MPT51 genes of both species localize close to the respective 85A component genes. Like the 85B component, the *M. leprae* 85A-MPT51 and 85C antigens are recognized by T cells from healthy contacts and leprosy patients.—Authors' Abstract

Dhople, A. M. and Ibanez, M. A. *In-vitro* activity of three new fluoroquinolones and synergy with ansamycins against *Mycobacterium leprae.* J. Antimicrob. Chemother. **32** (1993) 445–451.

The efficacy of three fluorinated quinolones, clinafloxacin (PD 127391), sparfloxacin (PD 131501) and PD 131628, either alone or in combination with rifampin/rifabutin, against *Mycobacterium leprae* was evaluated *in vitro* using two biochemical parameters to measure the metabolic activity of the organism. Clinafloxacin was found to be most effective with an MIC of 0.75 mg/L, followed by sparfloxacin (MIC 1.5 mg/L) and PD131628 (MIC 3.0 mg/L). When combined with rifampin each of the three quinolones were additive to the activity. However, when combined with rifabutin, both clinafloxacin and sparfloxacin demonstrated pronounced synergic activity. Incorporation of clinafloxacin and rifabutin in a multidrug therapy regimen is suggested.—Authors' Abstract

Finken, M., Kirschner, P., Meier, A., Wrede, A. and Bottger, E. C. Molecular basis of streptomycin resistance in *Mycobacterium tuberculosis*: alterations of the ribosomal protein S12 gene and point mutations within a functional 16S ribosomal RNA pseudoknot. Mol. Microbiol. **9** (1993) 1239–1246.

Multidrug-resistant strains of *Mycobacterium tuberculosis* have resulted in several recent outbreaks. Recognition of drug resistance is important both for treatment and to prevent further transmission. Here we use molecular biology techniques to study the basis of streptomycin resistance in single and multidrug-resistant *M. tuberculosis.* We demonstrate that streptomycin resistance is associated with mutations implicated in ribosomal resistance. The mutations found either lead to amino acid changes in ribosomal protein S12 or alter the primary structure of the 16S rRNA. The 16S rRNA region mutated perturbs a pseudoknot structure in a region which has been linked to ribosomal S12 protein.—Authors' Summary

Hackel, C. [Specific identification of *M. leprae* with the polymerase chain reaction technique.] Hansenol. Int. **15** (1990) 67–75. (in Portuguese)

The author describes the methodology based on the polymerase chain reaction (PCR) for the specific detection of *Mycobacterium leprae* DNA, by reviewing the approaches concerning the choice of oligonucleotide primers and the criteria of specificity and sensitivity required for a useful tool in clinical and experimental studies.—Author's English Abstract

Harvey, S. S., McKenzie, K. R., Roche, P. W. and Britton, W. J. Sequence and expression of the *Mycobacterium leprae dnaJ* gene. J. Gen. Microbiol. **139** (1993) 2003–2008.

Study of *Mycobacterium leprae,* the causative agent of leprosy, has been advanced by the isolation of genes encoding mycobacterial proteins including *dnaK* encoding the *M. leprae* 70-kDa heat-shock protein. The sequence downstream from *dnaK* revealed a second open reading frame coding for a protein of 389 amino acids with a calculated molecular mass of 41.2 kDa. Sequence analysis demonstrated significant DNA homology with the *dnaJ* gene of other organisms. High amino-acid sequence identity was obtained between the DnaJ protein of *M. leprae* and *M. tuberculosis* (89%) with significant divergence between the two occurring only at the C-terminal end. The expressed recombinant DnaJ protein had a molecular mass of 42 kDa.—Authors' Abstract

Heym, B., Zhang, Y., Poulet, S., Young, D. and Cole, S. T. Characterization of the *katG* gene encoding a catalase-peroxidase required for the isoniazid susceptibility of *Mycobacterium tuberculosis.* J. Bacteriol. **175** (1993) 4255–4259.

The isoniazid susceptibility of *Mycobacterium tuberculosis* is mediated by the product of the *katG* gene which encodes the heme-containing enzyme catalase-peroxidase. In this study, the chromosomal location of *katG* has been established and its nucleotide sequence has been determined so that the primary structure of catalase-peroxidase could be predicted. The *M. tuberculosis* enzyme is an 80,000-dalton protein containing several motifs characteristic of peroxidases and shows strong similarity to other bacterial catalase-peroxidases. Expression of the *katG* gene in *M. tuberculosis, M. smegmatis,* and *Escherichia coli* was demonstrated by Western blotting (immunoblotting). Homologous genes were detected in other mycobacteria, even those which are naturally insensitive to isoniazid.—Authors' Abstract

Ishaque, M. and Sticht-Groh, V. Investigations into the growth of *Mycobacterium leprae* in a medium with palmitic acid under different gaseous environments. Microbios **75** (1993) 171–179.

Low oxygen tension has often been considered important for the growth of *Mycobacterium leprae.* Palmitic acid has been suggested as the oxidizable substrate or the *in vitro* cultivation of leprosy bacilli. The combined effects of palmitic acid and various known gas mixtures on the *in vitro* growth of *M. leprae* were investigated. When palmitic acid was included in the medium an optimal growth in both liquid and solid media was obtained between 16 to 20 weeks of incubation under gas mixtures containing 2.5 or 5% O_2 and 5 or 10% CO_2 as well as air. The use of different gas mixtures is tedious, time consuming and laborious. Since the cultures incubated under air gave the same cell yield as obtained when incubated under optimal gas mixtures, air alone can be used for the *in vitro* cultivation trials of *M. leprae* when palmitic acid is included in the culture medium.—Authors' Abstract

Ishaque, M. and Sticht-Groh, V. Oxidation of insoluble palmitic acid and water soluble palmitic acid-methylated cyclodextrin complex by *Mycobacterium leprae* and *M. phlei.* Microbios **75** (1993) 107–115.

Palmitic acid could be a suitable substrate for the *in vitro* cultivation of *Mycobacterium leprae* but being insoluble in water it cannot be used effectively. Recently, dimethyl-beta-cyclodextrin complexed palmitic acid soluble in water has become available. Thus, oxidation of water-soluble and insoluble palmitic acid by the host-grown *M. leprae* and *in vitro*-grown *M. phlei* was investigated by measuring the rate of oxygen uptake. Insoluble and soluble palmitic acid was oxidized by *M. leprae* cell suspensions after a lag period of 6 and 2 hr, respectively. Soluble palmitic acid was oxidized by *M. phlei* without any lag period; whereas 60 min were required before insoluble palmitic acid was oxidized. The methylated-beta-cyclodextrin alone is an inert substance, and it had no inhibitory or stimulatory effect on the oxidation of soluble palmitic acid. Oxidation of soluble palmitic acid was markedly inhibited by some inhibitors of the respiratory chain. Results

indicate that oxidation of palmitic acid by *M. leprae* and *M. phlei* is mediated through the electron transport chain. Compared with insoluble palmitic acid, water-soluble palmitic acid has many advantages. Its use is recommended for metabolic studies, as well as in culture media used for *in vitro* cultivation trials of *M. leprae.*—Authors' Abstract

Jamil, S., Keer, J. T., Lucas, S. B., Dockrell, H. M., Chiang, T. J., Hussain, R. and Stoker, N. G. Use of polymerase chain reaction to assess efficacy of leprosy chemotherapy. Lancet **342** (1993) 264–268.

The assessment of chemotherapy efficacy in leprosy is difficult, since the only reliable method for determining whether the causative organism, *Mycobacterium leprae,* is viable depends on its growth in mouse foot pads. In an attempt to replace this expensive, time-consuming test, methods based on the polymerase chain reaction (PCR) have been developed. These methods depend on detection of DNA, which is more susceptible to degradation on cell death than are other cell components, so should be a more accurate indicator of viability. We have used a specific PCR assay to detect *M. leprae* DNA in skin biopsy samples from leprosy patients. By use of limiting dilution PCR (LD-PCR), the concentration of *M. leprae* DNA in the original sample could be measured. The DNA concentration was more closely correlated with the morphological index (derived from a staining technique that distinguishes morphologically intact and damaged bacteria) than with the number of bacteria visible [bacterial index, (BI) which counts both alive and dead bacteria]. In a longitudinal study of multibacillary patients on multidrug therapy, skin biopsy samples were collected before treatment and 3, 6, 12, and 24 months after the start of therapy. While the BI showed little or no change during treatment, the number of genomes detected by PCR fell sharply, in parallel with the MI.

We propose that PCR can be used as a rapid measure of *M. leprae* viability and that this approach can be used for monitoring individual leprosy patients and for assessment of existing and new regimens. The method may be applicable to other infectious diseases in which culture of the causative organism is slow or impossible.—Authors' Abstract

Kaur, I., Kaur, S., Sharma, V. K., Agnihotri, N., Vaishnavi, C. and Ganguly, N. K. Bacillaemia and *Mycobacterium leprae* cell wall antigen in paucibacillary leprosy. Indian J. Lepr. **65** (1993) 283–288.

A study was undertaken to estimate bacillemia and *Mycobacterium leprae* antigen detection in 54 paucibacillary leprosy patients (TT, BT). Acid-fast bacilli were detected in the blood of 14.8% patients of borderline tuberculoid (BT) leprosy. *M. leprae* antigen was demonstrated in 48.2% patients of BT leprosy. Slit-skin smears were negative in all these patients. At the end of treatment (6 months of WHO-MDT) all the follow-up blood samples were negative for both bacillemia and *M. leprae* antigen in the serum.—Authors' Abstract

Kirschner, P., Springer, B., Vogel, U., Meier, A., Wrede, A., Kiekenbeck, M., Bange, F.-C. and Bottger, E. C. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. J. Clin. Microbiol. **31** (1993) 2882–2889.

Clinical isolates of *Mycobacterium* spp. were identified by direct sequence determination of 16S rRNA gene fragments amplified by polymerase chain reaction (PCR). Identification was based on a hypervariable region within the 16S rRNA gene in which mycobacterial species are characterized by species-specific nucleotide sequences. A manually aligned data base including the signature sequences of 52 species of mycobacteria easily allowed rapid and correct identification. The results of this study demonstrate that PCR-mediated direct sequence determination can be used as a rapid and reliable method for the identification of mycobacteria in the clinical laboratory. In addition, the prompt recognition of previously undescribed species is now feasible.—Authors' Abstract

Lambrecht, R. S. and Collins, M. T. Inability to detect mycobactin in mycobacteria-infected tissues suggests an alternative iron acquisition mechanism by

mycobacteria *in vivo*. Microb. Pathogen. **14** (1993) 229–238.

Although most species of mycobacterium are capable of producing mycobactin, it is not known if conditions within the host allow for mycobactin synthesis or whether it even plays a role in iron acquisition *in vivo*. We employed the mycobactin-auxotroph, *Mycobacterium paratuberculosis*, in a bioassay to examine tissues from animals infected with either *M. tuberculosis*, *M. avium* or *M. paratuberculosis* for the presence of mycobactin or compounds which demonstrate mycobactin-like activity. Other iron-binding compounds, including purified siderophores from unrelated organisms and host iron-binding proteins were also evaluated in the bioassay for growth induction of *M. paratuberculosis* in the absence of mycobactin. Although mycobactin could be easily demonstrated in tissues artificially seeded with mycobacteria, no mycobactin could be detected in heavily infected tissues. None of the purified siderophores from unrelated microorganisms were found to support growth of *M. paratuberculosis* in the absence of mycobactin. Host iron-binding proteins (transferrin, lactoferrin, ferritin, hemin) also failed to induce growth in the bioassay at pH 6.8. However, when the pH was adjusted between 5–6.2, transferrin and lactoferrin promoted growth of *M. paratuberculosis* without mycobactin, probably as a result of the dissociation of iron rather than a specific interaction. We confirm that mycobacteria are incapable of iron uptake when iron is chelated to siderophores from unrelated organisms and conclude that mycobactin-mediated mechanisms of iron-acquisition by mycobacteria do not appear to have as significant a role *in vivo* as *in vitro*. In addition, evidence is presented that suggests iron-containing transferrin and lactoferrin at low pH may circumvent the need for mycobactin by *M. paratuberculosis*.—Authors' Abstract

Nair, J., Rouse, D. A., Bai, G.-H. and Morris, S. L. The *rpsL* gene and streptomycin resistance in single and multiple drug-resistant strains of *Mycobacterium tuberculosis*. Mol. Microbiol. **10** (1993) 521–527.

The recent emergence of indolent and rapidly fatal drug-resistant strains of *Mycobacterium tuberculosis* has renewed interest in defining the molecular mechanisms of drug resistance in the tubercle bacilli. In this report, we have examined the mechanism of resistance to streptomycin (Sm) in *M. tuberculosis* through the cloning and nucleotide sequence analysis of the gene encoding the ribosomal S12 protein (*rpsL* gene) from streptomycin-resistant strains and their streptomycin-sensitive parental strains. We have demonstrated that five singly Sm^R *M. tuberculosis* strains and an Sm^R isolate that has reduced sensitivity to multiple antibiotics have identical point mutations at codon 43 of the *rpsL* gene. Mutations at this same site confer Sm^R in *Escherichia coli*. In contrast, two other multiple drug-resistant *M. tuberculosis* strains that are resistant to Sm have *rpsL* genes that have the same nucleotide sequence as their drug-sensitive parent strains, suggesting that different resistant mechanisms are involved in these strains.—Authors' Summary

Nikaido, H., Kim, S.-H. and Rosenberg, E. Y. Physical organization of lipids in the cell wall of *Mycobacterium chelonae*. Mol. Microbiol. **8** (1993) 1025–1030.

Mycobacterial cell wall functions as an effective permeability barrier, making these bacteria resistant to most antibacterial agents. It has been assumed that this low permeability was due to the presence of a large amount of unusual lipids in the cell wall, but it was not known how these lipids are able to produce such an exceptional barrier. We report here the first experimental evidence on the physical arrangement of these lipids based on X-ray diffraction studies of purified *Mycobacterium chelonae* cell wall, a result suggesting that the hydrocarbon chains of the cell-wall lipids are arranged predominantly in a direction perpendicular to the cell wall surface, probably producing an asymmetric bilayer structure.—Authors' Summary

Patel, B. K. R., Banerjee, D. K. and Butcher, P. D. Determination of *Mycobacterium leprae* viability by polymerase chain reaction amplification of 71-kDa heat-shock

protein. (Letter) J. Infect. Dis. **168** (1993) 799–800.

Several attempts have been made to develop *in vitro* assays to measure the viability of *Mycobacterium leprae* and to screen antileprosy compounds. However, many of these assays were not reproducible, required very expensive equipment, or were not easy to perform. We have developed a method for the extraction and characterization of intact mRNA from slowly growing mycobacteria. We report an assay to determine the viability of *M. leprae* by cDNA-linked polymerase chain reaction (PCR) amplification of 71-kDa heat-shock protein (hsp) mRNA. The assay is based on the proposition that due to the short half-life (~2 min) of prokaryotic mRNA, dead bacilli will have either no mRNA or much reduced levels compared with viable bacilli.

The assay described here differentiates viable from dead leprosy bacilli on the basis of levels of short-lived mRNA. By using this technique, hsp mRNA can be detected from a minimum of 10^4–10^5 bacilli, a 100- to 1000-fold increase in sensitivity over that of the BACTEC 460 system. The method is superior in ease, speed, specificity, and sensitivity for the study of short-lived, low copy number mRNA transcripts. It can be completed in 1–2 days. The sensitivity of the assay could be further increased by using heat shock to increase the levels of hsp mRNA or by detection of as yet undefined high-abundance mRNA species.—From the Letter

Rinke de Wit, T. F., Clark-Curtiss, J. E., Abebe, F., Kolk, A. H. J., Janson, A. A. M., van Agterveld, M. and Thole, J. E. R. A *Mycobacterium leprae*-specific gene encoding an immunologically recognized 45-kDa protein. Mol. Microbiol. **10** (1993) 829–838.

By screening a *Mycobacterium leprae* lambda gt11 expression library with a serum from an Ethiopian lepromatous leprosy (LL) patient a clone was isolated (LL4) belonging to hybridization group III of a panel of previously isolated *M. leprae* clones. Members of this hybridization group encode a serologically recognized 45-kDa protein. The complete DNA sequences of the partially overlapping clones LL4 and L1 (hybridization group III) are presented, and these revealed the presence of an open reading frame (ORF) predicting a protein with a molecular size of 42,448 Da. Southern hybridizations on total genomic DNA of *M. leprae, M. tuberculosis* and eight atypical mycobacteria showed that the LL4 DNA fragment is specific for *M. leprae* DNA even under low-stringency conditions. The *M. leprae* specificity of LL4 DNA was further confirmed by the polymerase chain reaction using four different sets of primers. Western blotting analyses showed that the *M. leprae* 45-kDa protein is frequently recognized by antibodies from leprosy patients and that this recognition is specific since no antibodies could be detected in sera of tuberculosis patients. T-cell proliferation assays also demonstrated T-cell recognition by leprosy patients and healthy contacts of the *M. leprae* 45-kDa protein. The specificity of the LL4 DNA region and the 45-kDa antigen that is encoded by hybridization group III could provide unique tools for the development of *M. leprae*-specific immunological and DNA reagents.—Authors' Summary

Santos, A. R., Demiranda, A. B., Sarno, E. N., Suffys, P. N. and Degrace, W. M. Use of PCR-mediated amplification of *Mycobacterium leprae* DNA in different types of clinical samples for the diagnosis of leprosy. J. Med. Microbiol. **39** (1993) 298–304.

DNA of *Mycobacterium leprae*, obtained by a highly efficient nucleic acid extraction procedure, was used for standardization of the amplification of an *M. leprae*-specific repetitive sequence by use of the polymerase chain reaction (PCR). With pure DNA, *M. leprae*-specific amplification was obtained with as low as 100 ag (1 ag = 10^{-18} g) of target DNA, a quantity equal to about one-tenth of the bacterial genome. Optimal processing of different types of clinical samples such as biopsy material, blood and lymph fluid, from multibacillary leprosy patients, was studied. Simple freezing-boiling cycles in the presence of Triton X100, with some additional sample-specific modifications such as pretreatment with NaOH to

eliminate PCR inhibitors, were found to be sufficient to yield amplification of bacterial DNA in samples from paucibacillary patients. Clinical samples from 27 untreated leprosy patients, covering the various clinical forms of the disease, and with a bacterial index ranging from 5+ to 0, were collected and processed for PCR analysis. After hybridization of the amplified material with a specific sequence, 25 of 27 patients analyzed gave positive results for *M. leprae* in at least one of the samples. The potential of PCR for the diagnosis of leprosy is discussed.—Authors' Abstract

Siddiqi, S. H., Heifets, L. B., Cynamon, M. H., Hooper, N. M., Laszlo, A., Libonati, J. P., Lindholm-Levy, P. J. and Pearson, N. Rapid broth macrodilution method for determination of MICs for *Mycobacterium avium* isolates. J. Clin. Microbiol. **31** (1993) 2332–2338.

A multicenter study was done to investigate the accuracy and reproducibility of a method for determining the MICs of antimicrobial agents against the *Mycobacterium avium* complex in 7H12 broth with the BACTEC system. In phase I, with eight drugs and 10 strains, intralaboratory reproducibility was 95.7% to 100%, allowing a 1-dilution difference upon repeat testing. The results of phase II testing with 41 additional strains were consistent with those obtained in phase I, with good interlaboratory reproducibility. The radiometric method was validated by sampling and plating of the same broth cultures and determining, by the number of CFU per milliliter, the lowest drug concentration that inhibited more than 99% of the initial bacterial population. Three test concentrations of each drug and the tentative interpretation of results are proposed. Radiometric MIC determination has the potential to become the method of choice for clinical microbiology laboratories and evaluation of new agents for the treatment of *M. avium* infections, both pulmonary and disseminated.—Authors' Abstract

Telenti, A., Imboden, P., Marchesi, F., Schmidheini, T. and Bodmer, T. Direct, automated detection of rifampin-resistant *Mycobacterium tuberculosis* by polymerase chain reaction and single-strand conformation polymorphism analysis. Antimicrob. Agents Chemother. **37** (1993) 2054–2058.

A rapid screening test was recently established for the detection of mutations in the *rpoB* gene of *Mycobacterium tuberculosis,* a region identified as the locus for rifampin resistance (Rif^r). The detection method involved the amplification by polymerase chain reaction (PCR) of the Rif^r region and the identification of mutations by single-strand DNA conformation polymorphism analysis (SSCP) of the amplification products. Experience using two different PCR-SSCP formats for the evaluation of BACTEC cultures and sputum is presented here: the previously described manual procedure for the detection of radiolabeled amplification products and an automated SSCP by which fluorescein-labeled products were detected on a Pharmacia DNA sequencer apparatus. All 17 different Rif^r mutations known to date were consistently detected. PCR-SSCP could be used for the evaluation of minimally grown cultures (BACTEC 12B medium with a growth index of ≤ 100) and for direct screening of microscopically positive sputa with greater than 10 organisms per field (magnification, $\times 250$). Implementation of this technique could result in rapid detection of rifampin resistance in *M. tuberculosis,* a marker of multidrug-resistant tuberculosis.—Authors' Abstract

Vandervliet, G. M. E., Schukkink, R. A. F., Vangemen, B., Schepers, P. and Klatser, P. R. Nucleic acid sequence-based amplification (NASBA) for the identification of mycobacteria. J. Gen. Microbiol. **139** (1993) 2423–2429.

Nucleic acid sequence-based amplification (NASBA), an isothermal amplification technique for nucleic acids (NA), was investigated for the species-specific identification of mycobacteria. A set of primers was selected from a highly conserved region of the 16S rRNA sequence of mycobacteria sandwiching a variable sequence to perform amplification of mycobacterial RNA. Species-specific probes for the *Mycobacterium tuberculosis* complex, *M. avium-paratuberculosis, M. intracellulare* and *M. leprae* were

hybridized in solution with the amplified nucleic acids of 10 pathogenic mycobacteria and 11 closely related bacteria, as well as with human-derived NA in an enzyme-linked gel assay (ELGA). Each probe was shown to hybridize specifically to the amplified single-stranded RNA of the corresponding species. Thirty-two clinical isolates of *M. tuberculosis* strains from different parts of the world were correctly identified by NASBA using the *M. tuberculosis*-complex-specific probe. In combination with the ELGA, NASBA could identify mycobacteria rapidly, i.e., in less than 6 hr. The relative simplicity and rapidity of this technique makes it an attractive tool for species-specific identification of mycobacteria.—Authors' Abstract

Wu, W., *et al.* [Identification of cultures obtained from leprosy specimen.] China Lepr. J. **9** (1993) 18–21. (in Chinese)

This article reports the results of identifying 57 acid-fast bacilli (AFB) cultures isolated from clinical specimens by using traditional methods (TM, including mainly biochemical and cultural properties) and modern ELISA with monoclonal antibody (McAb-ELISA) and nested primer gene amplification assay (NPGAA). The representatives (M. A1, A7, A15, A21 and A22) of AFB cultures isolated from human lepromas were identified as new species different from known standard mycobacteria by TM, while not identical to *M. leprae* by McAb-ELISA and NPGAA. As for the representatives (M. S17, S1, S2, S22, S7, S25), M. S17 was found to be identical to *M. scrofulaceum* by using TM only, others (M. S1, S2, S22, S7, S23) were identified as mycobacteria close to *M. tuberculosis* and different from *M. leprae* with TM and McAb-ELISA, as *M. tuberculosis* with NPGAA. From these studies, it is considered that the TM and modern methods (MM) are all very useful in identifying mycobacteria either alone or integrately, although MM was much more sensitive and specific to detection and identification of mycobacteria than TM, especially integration of TM and MM could not only complement each other, but also become more complete. The way of selecting and using these methods alone or integrately depends on the purposes and practical needs.—Authors' English Abstract

Epidemiology and Prevention

Andrade, V. L. G., Sabroza, P. C., Castro, A. J. W. and Motta, C. P. Leprosy spread in urban area: Part I: Epidemiological characteristics of an endemic urban area for leprosy; the county of São Gonçalo, Rio de Janeiro State, Brazil. Hansenol. Int. **15** (1990) 24–45.

Among the needs of deepening the epidemiological inquiry studies of leprosy diffusion in urban areas, the evaluation of the Local Information System stands out as the base instrument to carry out an epidemiological and operational analysis. The information system was utilized also as base to the populational planning studies. In the present paper, the achievement of the recorded cases mapping in three sanitary units in respective copyhold sectors, as well as the analysis of the specific epidemiological and operational indicators, permitted the characterization of the São Bonçalo county as urban endemic area for leprosy, the delimitation of the foci and the inquiry planning. Starting with the specific indicators, according to age, sex, clinical form in the case register date, it was verified a higher detection rate in women than in men, as well as an increase of the tendency of the tuberculoid forms. In the epidemiological inquiry conducted during 85 days, 926 dwellings were visited with interview, physical examination, anthropometric measurings and soluble antigen (SA) application. Such inquiry was conducted by a staff of health professionals and specially trained and standardized community members. The produced results confirmed that in the copyhold sectors without registered cases no case was found and that the domiciliary visits on Sunday do not present a superior productivity to the other days of the week. The

whole of the findings lead one to conclude that the Local Information System is a valuable instrument for the description of the endemic characteristics in this county and file a construction adequate to the planning of populational studies.—Authors' Abstract

Andrade, V. L. G., Sabroza, P. C., Castro, A. J. W., Motta, C. P. and Araujo, A. J. G. Leprosy spread in urban area: Part II: Reactivity of soluble antigen (SA) in three different groups of leprosy contacts in São Gonçalo, Rio de Janeiro State, Brazil. Hansenol. Int. **15** (1990) 46–57.

In three different groups of leprosy contacts there were observed results of intradermal reaction to the soluble antigen (SA). In urban area endemic for leprosy (prevalence rate of 2.87 per 1000 inhabitants) there were studied three groups: 1) intradomiciliary contacts or living together with a case for more than 1 year; 2) neighboring contacts of extradomiciliary ones; 3) inhabitants in the areas far from the focus. Thirty-nine percent of the 1569 intradermal reactions were positive. It was demonstrated an association between the positiveness of the intradermal reaction and the proximity of the infection source, with the age and the time of residence in the same dwelling. The SA was considered a good instrument for the identification of the groups of risk for leprosy, although the findings do not quantify its predicting value in the individual diagnosis of the infection.—Authors' Abstract

Baker, D. M., Nguyen Van Tam, J. S. and Smith, S. J. Protective efficacy of BCG vaccine against leprosy in southern Malawi. Epidemiol. Infect. **111** (1993) 21–25.

This paper describes a matched case-control study to determine the efficacy of BCG vaccine in preventing the occurrence of leprosy in southern Malawi, a previously unstudied area. The BCG immunization rate among 145 individuals with leprosy was 44.8%, compared to 62.5% in 290 matched controls. The protective efficacy of BCG vaccine against leprosy in this region was estimated to be 63.6%; smallpox immunization had no effect. These findings support the view that BCG vaccine should be considered as a control measure in areas where leprosy is endemic.—Authors' Abstract

Boerrigter, G. and Ponnighaus, J. M. Does the introduction of WHO-MDT influence trends in the incidence of leprosy?—the Malawian experience. Lepr. Rev. **64** (1993) 227–235.

There has been an average annual decline in detection rates of all types of leprosy in Malawi of around 11.6% between 1977 and 1991. There was no obvious acceleration or slowing down of this decline following the introduction of WHO/MDT in 1983–1984. Disability ratios stayed at the same level of about 11% during the 15 years covered by this paper, suggesting that patients did not self-report earlier after 1983–1984 which might have masked an underlying accelerated decline in detection rates. Thus, it is concluded that the influence of WHO/MDT on the pattern of leprosy over a period of time, in a country like Malawi, is so far not noticeably different from any influence dapsone monotherapy might have had.—Authors' Summary

Kaneko, K. A., Zambon, V. D. and Pedrazzani, E. S. [New cases of hanseniasis in the São Carlos, SP, (Brazil) Region, 1983–1988.] Hansenol. Int. **15** (1990) 5–15. (in Portuguese)

This paper presents an analysis of leprosy situation at São Carlos region between 1983 and 1988. Employing official data, a general characterization of 121 new cases is shown. Leprosy occurs more frequently among married people, whites, more than 20 years old, a low school level and urban population. It can be observed two serious troubles: 1) health services are inefficient in the detection of new cases; 2) population is not informed about this disease, that there is a long time between initial symptoms and diagnosis.—Authors' English Abstract

Klatser, P. R., van Beers, S., Madjid, B., Day, R. and de Wit, M. Y. L. Detection of *Mycobacterium leprae* nasal carriers in populations for which leprosy is endemic. J. Clin. Microbiol. **31** (1993) 2947–2951.

In order to better understand the role of *Mycobacterium leprae* nasal carriage in the

maintenance of infection reservoirs and transmission of leprosy, we applied a polymerase chain reaction (PCR) that detected a 531-bp fragment of the *pra* gene of *M. leprae* on nasal swab specimens collected through a total population survey from individuals living in an area in which leprosy is endemic. Among the total tested population of 1228 people, 7.8% were found to be PCR positive. PCR positivity was shown to be randomly distributed among the population for which leprosy is endemic. No association was observed between PCR positivity, age, or sex. The observed distribution of PCR positivity among households of different sizes confirmed the expected values, with the exception of two households, each with three people with PCR-positive nasal swab specimens. Although nasal carriage does not necessarily imply infection or excretion of bacilli, the finding of nasal carriage supports the theory of a disseminated occurrence of *M. leprae* in populations for which leprosy is endemic.—Authors' Abstract

Lombardi, C. Hanseniasis control in São Paulo State, Brazil. Hansenol. Int. **14** (1989) 14–31.

Socioeconomic and sanitary characteristics of São Paulo State, where the Unified and Decentralized Health System (SUDS) is being developed, are described. SUDS basic strategy is the municipalization of primary health care. The Hanseniasis Control Program (GEPRO-hanseniasis), included in this context, is analyzed under the point of view of administrative connections, objectives and performance. Special programs aiming at the absorption of new technology, such as multidrug therapy and the early serological diagnosis of the disease, are also studied.—Author's Abstract

Shanker Narayan, N. P., Muthusamy, P., Louis, S. and Ramu, G. Sample survey of leprosy after three years of MDT in Bhavani taluk of Periyar District, Tamil Nadu (India). Indian J. Lepr. **65** (1993) 289–295.

A sample survey of Bhavani taluk was undertaken in March 1992, 3 years after the introduction of MDT. Ten percent of the population was taken for the sample. A population of 45,781 was enumerated and 41,554 were examined. The three sectors were stratified according to the prevalence rate and classifying the villages by the size of the population. Villages were selected by random sampling. The sample survey detected 288 new cases of leprosy of which 16 (5.55%) were bacteriologically positive for acid-fast bacilli. The child rate was 13.54% among new cases. According to the sample survey the current prevalence rate per 1000 population was 9.07 (with a new case detection rate of 6.93/1000 population), much higher than that derived from program data (prevalence rate 3.45) and the expected tenfold reduction of prevalence under MDT. Independent sample surveys of NLEP units after 3 to 5 years of implementation of MDT will help to assess deficiencies in the program and enable us to take remedial measures.—Authors' Abstract

Rehabilitation

de Oliveira, M. H. P. [Emotional reactions of hanseniasis patients with physical deformities.] Hansenol. Int. **15** (1990) 16–23. (in Portuguese)

This work's goal was gathering information to have an assessment of the leprosy patient's emotional reactions. Those patients have physical deformity and receive assistance at a health center in Ribeirão Preto. Regardless of their sex, age, treatment time and the clinical form of the disease, 22 patients were interviewed through an instrument and a qualitative analysis of the recorded speeches, by the question: "How do you see (face) your physical deformity?" The analysis of those speeches pointed out some emotional alterations reflecting several reactions such as: fear, disgust, loneliness, grief, aggressiveness, anger, family and social rejection, worries about the future, inferiority complex, etc. Those reactions

must be identified and understood by all the health staff in order to support the patients, be clear about their living situation, trying to clear their minds of false concepts and taboos which are still persistent relating to the disease.—Author's English Abstract

Jiang, C., *et al.* [Demands of leprosy patients with disability.] China Lepr. J. **9** (1993) 9–13. (in Chinese)

The authors surveyed the demand of 1031 leprosy patients and cured cases in Yangzhou City, Jiangsu Province. Among them 95.8% had disability Grade II or III and 69.1% were cured. The demand on society in those with severe disability is higher than in those with lesser damage. The main need is to increase the level of life in the former and to improve disability among the latter; 78.5% of them are willing to live in a household and 83.7% of active patients prefer to be treated at a clinic. Those who hope to life or be treated in a leprosy hospital are 11% of the 1031 persons, 16.3% of active patients, 78.1% of inpatients, 19.7% of those who lead a solitary life and 4% of those who live in households. This suggests that the persons with severer disability are more likely to live in a hospital than those with slighter disability. Outpatients hope to be treated secretly in 25.1% and at a clinic in 11.2%. The cured hope to be secretly followed up in 29.5% and to attend a clinic in 33.2%.—Authors' English Abstract

Saha, S. P. and Das, K. K. Disability pattern amongst leprosy cases in an urban area (Calcutta). Indian J. Lepr. **65** (1993) 305–314.

In a retrospective study of 1264 leprosy cases, registered during 1987–1992, 282 were found to have disabilities giving a disability rate (DR) of 22.31% and 150 of them were also found to have deformities, giving a deformity rate of 11.9%. Mean disability index (DI) was found to be 1.17. Disability rate (DR) significantly increased with age and the highest rate was 52.75% in lepromatous (L) cases, followed by 27.51% in borderline (N?L) and only 4.53% in nonlepromatous (N) cases. L cases had the highest deformity rate (22.25%) and N cases had the lowest DR (2.23%). DI was highest (1.46) in L, and lowest (0.52) in N cases. Males had significantly higher DR (27.2%) compared to females (13.0%). Deformity in hands (42.55%) was more common than in feet (22.70%). Increasing trend of DI was noticed with increasing duration of disease in L and N?L types. The number of nerves involved was high (4.72) in L cases compared to other types. DI was highest (1.25) in patients engaged in occupations involving hard work.—Authors' Abstract

Yi, S., *et al.* [Psychology of leprosy patients.] China Lepr. J. **9** (1993) 80–83. (in Chinese)

The influences of sex, age, educational level, disease duration, disability and having or not close relatives on their mind in 71 patients with leprosy have been analyzed by using the disease self-estimate table of SCL-90. The results showed their suffering levels to be higher than those in healthy persons and the total mean score of psychological response was equal to 105.55 ± 57.21. The degree of disability has made a direct impact on the patient's mind. As compared with the patients living at home, the patients isolated in leprosaria without close relatives have higher scores in compulsion, depression, phobic anxiety, bigoted ideas and psychosis. The longer the disease duration, the more severe the lost and phobic feeling and hostility. The illiterate patients have more serious lost and phobic feeling. According to ten factors in SCL-90, the most sensitive ones are interpersonal sensitivity, depression and phobic anxiety, and the next ones are compulsion, anxiety and hostility. After they firmly believed the diagnosis, a few patients began excessively drinking, became superstitious, gamblesome, hostile and even tending to suicide. Forty-eight of the 74 cases had suicide intentions and four committed suicide themselves (without completion). The authors point out that for the psychological health of leprosy patients it will be necessary to obtain the support the public in removing discrimination against the patients through popular health education and to find and treat the patients as early as possible in the interests of prevention of the disability.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Abe, C., Hirano, K., Wada, M., Kazumi, Y., Takahashi, M., Fukasawa, Y., Yoshimura, T., Miyagi, C. and Goto, S. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. J. Clin. Microbiol. **31** (1993) 3270–3274.

The polymerase chain reaction (PCR) using oligonucleotides based on the repetitive sequence (IS*986*) of *Mycobacterium tuberculosis* as a primer and the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test (MTD), which combines an *M. tuberculosis* rRNA amplification method with the hybridization protection assay format, were evaluated for detection of *M. tuberculosis* in clinical samples. The detection limits of these two assay systems based on nucleic acid amplification for cultured *M. tuberculosis* were less than 10 cells per reaction. A total of 135 sputum specimens were examined by the two assay systems. The PCR and the MTD systems for detection of *M. tuberculosis* gave overall positivity rates of 84.2% (32 of 38) and 91.9% (34 of 37), respectively, as compared with 71.9% (23 of 32) by smear and 96.9% (31 of 32) by culture in the liquid medium MB-Check. Procedures for sample preparation used in the two methods were different. Although the sensitivities of the PCR and MTD appeared to be similar to that of culture with the MB-Check system, the two methods based on nucleic acid amplification should be very useful for rapid detection of *M. tuberculosis* infections without the long time required for culture of *M. tuberculosis.*—Authors' Abstract

Arruda, S., Bomfim, G., Knights, R., Huima-Byron, T. and Riley, L. W. Cloning of a *M. tuberculosis* DNA fragment associated with entry and survival inside cells. Science **261** (1993) 1454–1457.

Mycobacterium tuberculosis infects one third of the world's human population. This widespread infection depends on the organism's ability to escape host defenses by gaining entry and surviving inside the macrophage. DNA sequences of *M. tuberculosis* have been cloned; these confer on a nonpathogenic *Escherichia coli* strain an ability to invade HeLa cells, augment macrophage phagocytosis, and survive for at least 24 hours inside the human macrophage. This capacity to gain entry into mammalian cells and survive inside the macrophage was localized to two distinct loci on the cloned *M. tuberculosis* DNA fragment.—Authors' Abstract

Atra, E. and Sato, E. I. Treatment of the cutaneous lesions of systemic lupus erythematosus with thalidomide. Clin. Exp. Rheumatol. **11** (1993) 487–493.

Twenty-three patients with systemic lupus erythematosus (SLE) and cutaneous lesions not responsive to chloroquine, photoprotectors and corticosteroid in doses < 0.5 mg/kg/day were treated with thalidomide 300 mg/day. Three patients presented side effects and had to discontinue treatment. Eighteen of the remaining 20 patients (90%) had complete remission of the cutaneous lesions and 2 had partial improvement. Another important parameter of improvement was a reduction in the average prednisone dose required from 40.5 mg/day to 17.4 mg/day. The most frequent side effects were drowsiness in 52% of cases and abdominal distention in 22%. These symptoms were reversed by dose reductions in all but one patient. Thalidomide was shown to be efficient in the treatment of cutaneous lesions unresponsive to more usual treatments.—Authors' Abstract

Barnes, P. F. and Barrows, S. A. Tuberculosis in the 1990s. Ann. Intern. Med. **119** (1993) 400–410.

Recent increases in tuberculosis morbidity in the United States are concentrated in racial and ethnic minorities, the foreign-born, and persons with human immunodeficiency virus infection. Amplification of *Mycobacterium tuberculosis* DNA by polymerase chain reaction allows rapid diagnosis of tuberculosis, and "DNA fingerprinting" of individual *M. tuberculosis* strains allows delineation of patterns of tu-

berculosis transmission. These techniques are available in research laboratories and are promising clinical tools for the future. Treatment regimens for drug-susceptible tuberculosis yield cure rates of more than 95%. Failure to ensure compliance with antituberculosis medications has resulted in an increasing prevalence of multiple-drug-resistant tuberculosis that responds poorly to therapy. Guidelines for isoniazid chemoprophylaxis have been modified in the past 5 years and are summarized. Control of tuberculosis in the United States will require improved implementation of established techniques to diagnose, treat, and prevent tuberculosis, with renewed emphasis on ensuring compliance with therapy.—Authors' Abstract

Bessesen, M. T., Shlay, J., Stonevenohr, B., Cohn, D. L. and Reeves, R. R. Disseminated *Mycobacterium genavense* infection—clinical and microbiological features and response to therapy—short communication. AIDS 7 (1993) 1357–1361.

Mycobacterium genavense is a newly described pathogen that causes disseminated infection in AIDS. It is difficult to detect and identify due to its slow growth and fastidious nature. There is little information available about therapy for this new pathogen. We describe clinical and laboratory features and response to therapy in four patients with advanced AIDS complicated by disseminated *M. genavense* infection from Denver, Colorado, U.S.A.

Retrospective analysis was made of four cases identified in an AIDS clinic affiliated with a municipal hospital in Denver, Colorado. Clinical samples were inoculated onto BACTEC 12B, Lowenstein-Jensen, and Middlebrook 7H11 media. The clinical features mimicked those of disseminated *M. avium* complex infection, with invasion of liver, spleen and lymph nodes with acid-fast bacilli (AFB). Acid-fast smears of blood and lymph nodes were positive; there was a modest increase in the growth index in BACTEC broth and tiny colonies appeared on Middlebrook agar. Patients were treated with combinations of antimycobacterial agents. Blood smears and cultures reverted to negative in treated patients. The best clinical response was associated with clarithromycin therapy.

Disseminated disease due to *M. genavense* should be suspected among patients with the clinical presentation of disseminated *M. avium* complex infection and low-growth index on BACTEC cultures for AFB. The diagnosis of *M. genavense* may be facilitated by performing acid-fast stains of samples from BACTEC bottles in such individuals. Clarithromycin therapy is associated with clinical improvement and clearance of bacteremia.—Authors' Abstract

Bottger, E. C., Hirschel, B. and Coyle, M. B. *Mycobacterium genavense* sp. nov. Int. J. System. Bacteriol. **43** (1993) 841–843.

Strains of a suggested novel type of mycobacterium have been repeatedly isolated from patients with AIDS. We summarize the results of tests performed to determine enzymatic activities and metabolic properties, the results of fatty acid analyses, and the results of a comparative 16S rRNA sequence determination. We propose formally that this organism represents new species, *Mycobacterium genavense.* The type strain is strain 2289, a culture of which has been deposited in the American Type Culture Collection as strain ATCC 51234.—Authors' Abstract

Chatterjee, D., Khoo, K. H., McNeil, M. R., Dell, A., Morris, H. R. and Brennan, P. J. Structural definition of the nonreducing termini of mannose-capped LAM from *Mycobacterium tuberculosis* through selective enzymatic degradation and fast atom-bombardment mass-spectrometry. Glycobiology **3** (1993) 497–506.

The application of extracellular arabinases from a *Cellulomonas* sp. and fast atom bombardment-mass spectrometry (FAB-MS) provided new insight into the structure of lipoarabinomannan (LAM) of *Mycobacterium tuberculosis,* a key molecule in the pathogenesis and physiology of the tubercle bacillus. Previously, the nonreducing arabinan ends of LAM from the virulent (Erdman) strain of *M. tuberculosis* were shown to be "capped" by short (alpha 1 → 2)-linked mannopyranose (Manp)-containing oligosaccharides, a product called ManLAM. The

structural relationship between these Manp units and the underlying arabinofuranose (Araf)-containing arabinan was examined by digesting ManLAM from *M. tuberculosis* Erdman with the *Cellulomonas* enzyme, resolving fragments by various means and subjecting the derivatized oligoglycosylalditols to FAB-MS. The sequences Manp2-Araf4, Manp3Araf4 and Manp1-6Araf6 were recognized as the major terminal motifs. Upon complete structural definition, all of the Ara6-containing products were shown to be based on a 3,5-linked branched Araf unit; whereas those containing Ara4 were linear. Minor non-mannosylated terminal arrangements containing Ara4-6, branched, linear and cyclical, were also recognized. In addition, the mannan "core" of ManLAM was isolated from enzyme digests and shown to contain segments of the phosphatidylinositol anchor and a "stub" of the arabinan side-chain in the form of a "linker" alpha-Araf-(1-5)-Araf unit attached to C-2, apparently of the penultimate 2,6-linked Manp residue. The structural unravelling of this complex molecule further substantiates the case for structural and biological similarities to the enterobacterial lipopolysaccharides/lipoglycans and other important "capped" lipooligomers such as the lipooligosaccharides of *Neisseria* species and the lipophosphoglycan of *Leishmania* promastigotes.—Authors' Abstract

Cheng, S. H., Walker, K. B., Lowrie, D. B., Mitchison, D. A., Swamy, R., Datta, M. and Prabhakar, R. Monocyte antimycobacterial activity before and after *Mycobacterium bovis* BCG vaccination in Chingleput, India, and London, U.K. Infect. Immun. **61** (1993) 4501–4503.

Monocytes from purified protein derivative S Mantoux-negative children and young adults inhibited intracellular growth of *Mycobacterium microti* more in Chingleput than in London. *M. bovis* BCG vaccination did not enhance bacteriostasis with the Indians but did so with the Londoners. No evidence was found for involvement of cytokines such as macrophage-activating factor and granulocyte macrophage colony-stimulating factor in the differences.—Authors' Abstract

Chevrel-Dellagi, D., Abderrahman, A., Haltiti, R., Koubaji, H., Gicquel, B. and Dellagi, K. Large-scale DNA fingerprinting of *Mycobacterium tuberculosis* strains as a tool for epidemiological studies of tuberculosis. J. Clin. Microbiol. **31** (1993) 2446–2450.

We conducted a large-scale DNA fingerprinting analysis of *Mycobacterium tuberculosis* strains in a country in which tuberculosis is endemic (Tunisia) in order to evaluate the importance of microepidemics in the maintenance of the disease within the population. The genetic polymorphisms of 201 strains of *M. tuberculosis* isolated from 196 unrelated patients living in four districts of northern Tunisia during a 3-year period were studied by restriction fragment length polymorphism (RFLP) analysis by using the insertion sequence IS*6110* as a probe. Seventy-three strains isolated from 68 patients living in the districts of Tunis, Nabeul, and Jendouba generated 67 different RFLPs, indicating a high degree of polymorphism of the *M. tuberculosis* strains within these areas. In contrast, the 128 strains isolated from individuals in the district of Menzel Bourguiba appeared much less heterogeneous since they often generated identical or very similar fingerprints. Seventeen of 29 cases (58%) of active tuberculosis in the city of Menzel Bourguiba could be traced to as few as four *M. tuberculosis* strains. These results indicate the persistence of underestimated microepidemics in this region. The RFLP typing of a large number of randomly collected strains provides a general picture of the strains involved in tuberculosis. The systematic study of limited areas where tuberculosis is endemic can provide evidence for the existence of persisting epidemics. This stresses the different problems which remain to be solved in order to improve the control of tuberculosis.—Authors' Abstract

Chugh, I. B., Vinayak, V. K. and Khuller, G. K. Host response to mycobacterial cell wall subunit during experimental tuberculosis in mice. Folia Microbiol. **38** (1993) 345–348.

The cell-wall protein-peptidoglycan complex (CW-PPC) of *Mycobacterium tuber-*

culosis, an immunologically potent component, was used to study the correlation between immune response and *in vivo* bacterial multiplication in the course of experimental tuberculosis infection in mice. Antibodies to CW-PPC were detected only after 7 weeks of infection with *M. tuberculosis* H37Rv and afterward no significant change was seen throughout the experiment. Delayed-type hypersensitivity (DTH) to CW-PPC showed a gradual increase from the fifth week onward with a maximum during the 12th week after infection which did not change significantly afterward. The increased immune response in the course of infection correlated well with the multiplication rate of bacilli in the lungs. These results indicate a role of CW-PPC in antituberculous immunity.—Authors' Abstract

Clarridge, J. E., Shawar, R. M., Shinnick, T. M. and Plikaytis, B. B. Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. J. Clin. Microbiol. **31** (1993) 2049–2056.

We investigated the use of DNA amplification by the polymerase chain reaction (PCR) for detection of *Mycobacterium tuberculosis* from clinical specimens. Two thirds of each sample was processed for smear and culture by standard methods, and one third was submitted for DNA extraction, amplification of a 317-bp segment within the insertion element IS6110, and detection by agarose gel electrophoresis, hybridization, or both. DNA was prepared from over 5000 samples, with 623 samples being culture positive for acid-fast bacilli. Of 218 specimens that were identified as *M. tuberculosis,* 181 (85%) were positive by PCR. In the *M. tuberculosis* culture-positive group, PCR was positive for 136 of 145 (94%) and 45 of 73 (62%) of the fluorochrome smear-positive and -negative specimens, respectively. Of 948 specimens that were either culture positive for mycobacteria other than *M. tuberculosis* or culture negative, 937 specimens were negative by PCR and 11 (1%) specimens initially appeared to be false-positive for *M. tuberculosis.* The reasons for discrepant results varied; some errors were traced to the presence of an inhibitor in the specimen (7.3% in unselected specimens), nucleic acid contamination, low numbers of organisms in the specimen, antituberculosis therapy, and possible low-level nonspecific hybridization. In comparison with culture, the sensitivity, specificity, and positive predictive values were 83.5%, 99.0%, and 94.2%, respectively, for PCR. When PCR was corrected for DNA contamination, the presence of inhibitor, and culture-negative disease, the values became 86.1%, 99.7%, and 98.4%, respectively. If the results for multiple specimens submitted from the same patient are considered, no patient who had three or more sputum specimens tested would have been misdiagnosed.—Authors' Abstract

Coronado, V. G., Becksaque, C. M., Hutton, M. D., Davis, B. J., Nicholas, P., Villareal, C., Woodley, C. L., Kilburn, J. O., Crawford, J. T., Frieden, T. R., Sinkowitz, R. L. and Jarvis, W. R. Transmission of multidrug-resistant *Mycobacterium tuberculosis* among persons with human immunodeficiency virus infection in an urban hospital—epidemiologic and restriction fragment length polymorphism analysis. J. Infect. Dis. **168** (1993) 1052–1055.

From January 1990 to December 1991, 16 patients with multidrug-resistant tuberculosis (MDR-TB) caused by *Mycobacterium tuberculosis* resistant to isoniazid, rifampin, and streptomycin were diagnosed at Elmhurst Hospital. Compared with other TB patients, MDR-TB patients were more likely to have human immunodeficiency virus (HIV) infection (14/16 vs. 21/204, $p < 0.001$) and a prior admission (10/16 vs. 3/204, $p < 0.001$). HIV-infected patients hospitalized for > 10 days within three rooms of an infectious MDR-TB patient had higher risk of acquiring MDR-TB than did HIV-infected patients with shorter hospitalizations or locations further from the MDR-TB patient(s) (6/28 vs. 2/90, $p < 0.001$). Isolates of 6 of 8 MDR-TB patients in a chain of transmission were identical by restriction fragment length polymorphism DNA typing. Ambulation on the wards of inadequately masked TB patients and lack of negative pressure in isolation rooms probably facilitated transmission. This re-

port documents nosocomial transmission of MDR-TB and underscores the need for effective isolation practices and facilities in health care institutions.—Authors' Abstract

Dechastellier, C., Frehel, C., Offredo, C. and Skamene, E. Implication of phagosome-lysosome fusion in restriction of *Mycobacterium avium* growth in bone marrow macrophages from genetically resistant mice. Infect. Immun. **61** (1993) 3775–3784.

The ability of the host to resist infection to a variety of intracellular pathogens, including mycobacteria, is strongly dependent upon the expression of the Bcg gene. Mouse strains which express the resistance phenotype [Bcg(r)] restrict bacterial growth, whereas susceptible strains [Bcg(s)] allow bacterial growth. Expression of the Bcg allele is known to influence the priming of host macrophages for bactericidal function. In the present work, bone-marrow-derived macrophages from congenic BALB/c [Bcg(s)] and C.D2 [BALB/c.Bcg(r)] mice were infected with the virulent strain *Mycobacterium avium* TMC 724 to define the mechanism involved in growth restriction of *M. avium*. By combining CFU measurements and ultrastructural analyses, we show that growth of this bacterium is restricted in marrow macrophages from resistant mice. Using acid phosphatase as a lysosomal marker, we provide evidence that the hydrolytic activity of macrophages, as measured by the capacity of lysosomes to fuse with and transfer active hydrolytic enzymes to phagosomes in which *M. avium* resides, is an expression of the Bcg gene and that this phenomenon is a key antibacterial activity responsible for growth restriction of *M. avium*: a) the percentage of phagosome-lysosome fusions was twice as high in Bcg(r) macrophages as in Bcg(s) macrophages, and b) the percentage of intact viable bacteria residing in acid phosphatase-negative phagosomes was twice as low in Bcg(r) macrophages as in their Bcg(s) counterparts. These differences are not due to a lower activity of the enzyme in Bcg(r) macrophages. The mechanism by which the Bcg gene exerts control over phagolysosomal fusion is discussed.—Authors' Abstract

Dolan, P. J., Raviglione, M. C. and Kochi, A. Estimates of future global tuberculosis morbidity and mortality. MMWR **42** (1993) 961–963.

This report uses tuberculosis (TB) notification data (i.e., cases reported to the ministries of health and collected by the World Health Organization [WHO]) to estimate the future global public health impact of TB and assesses the present and future contribution of HIV infection to TB.

During 1990, an estimated 7.5 million incident cases of TB occurred worldwide. Approximately 4.9 million cases (66%) occurred in the Southeast Asian and Western Pacific regions; India (2.1 million), China (1.3 million), and Indonesia (0.4 million) accounted for the largest number of cases. By 2005, the incidence of TB may increase to 11.9 million cases per year—an increase of 58% over 1990. Demographic factors (e.g., population growth and changes in the age structure of populations) will account for 77% of the predicted increase in incidence; epidemiologic factors (e.g., changes in incidence rates associated with the HIV epidemic) will account for 23%. For example, incidence rates for Africa may increase by 10 additional cases per 100,000 population per year during 1990–2005, primarily because of the HIV epidemic. In the Southeast Asian, Western Pacific, Eastern Mediterranean, and American regions, age-specific incidence rates are expected to decline during 1990–2005; in comparison, age-specific rates in Eastern Europe, Western Europe, and other industrialized countries may remain stable. However, because of population growth, the total number of new cases in these regions will continue to increase.

The estimated impact of HIV infection on TB incidence was based on reported HIV seroprevalence data among patients with TB, assumed changes in HIV seroprevalence by region through 2000, and the estimation that 95% of HIV-associated TB cases are attributable to HIV infection. For 1990, an estimated 4.2% of all incident TB cases were attributable to HIV infection. This proportion may increase to 8.4% in 1995 and to 13.8% by 2000, when more than 1.4 million cases will be attributable to HIV infection. During 1990–1999, an estimated 88.2 million persons will develop

TB; 8 million of those cases will be attributable to HIV infection.

For 1990, an estimated 2.5 million deaths occurred from TB, of which 116,000 were associated with HIV infection. In 2000, an estimated 3.5 million TB deaths will occur (39% more than in 1990), and approximately 0.5 million will be associated with HIV infection. Almost half of these HIV-associated deaths will occur in sub-Saharan Africa. During 1990–1999, an estimated 30 million persons will die from TB; approximately 3 million of those deaths will be associated with HIV infection. In Southeast Asia, 12.3 million deaths from TB will occur during the decade, of which approximately 1 million will be associated with HIV infection. Nearly 6 million TB deaths are projected in sub-Saharan Africa, of which approximately 1.5 million will be associated with HIV infection.—From the Report

Donald, P. R., Victor, T. C., Jordaan, A. M., Schoeman, J. F. and Van Helden, P. D. Polymerase chain reaction in the diagnosis of tuberculous meningitis. Scand. J. Infect. Dis. **25** (1993) 613–617.

Forty-three cerebrospinal fluid (CSF) specimens obtained from 20 children with tuberculous meningitis (TBM) at varying times during the first month of treatment were examined by polymerase chain reaction (PCR) for the presence of *Mycobacterium tuberculosis* DNA. Overall 27 CSF specimens (63%) from 16 patients (80%) gave ≥ 1 positive results and positive results were obtained from CSF specimens throughout the first 4 weeks of therapy. Nine CSF specimens (21%) gave a doubtful result (only 1 of duplicate determinations positive) and 7 (16%) a negative result. CSF from patients with suspected TBM should be submitted for PCR evaluation and positive results may be obtained up to at least 4 weeks after the start of treatment.—Authors' Abstract

Ellard, G. A., Humphries, M. J. and Allen, B. W. Cerebrospinal fluid drug concentrations and the treatment of tuberculous meningitis. Am. Rev. Respir. Dis. **148** (1993) 650–655.

Tuberculous meningitis is a very serious form of tuberculosis. In the absence of randomized controlled trials of alternative treatment regimens, its management depends on employing potent drugs that penetrate well into the cerebrospinal fluid (CSF). The penetration of isoniazid, rifampin, and streptomycin into the CSF of 27 Chinese patients was studied using fluorimetric and microbiologic procedures. Isoniazid rapidly diffused into the CSF, peak concentrations in excess of 3 mg/L, or over 30 times its minimal inhibitory concentration (MIC) against *Mycobacterium tuberculosis* being attained within 4 hr. In contrast, rifampin and streptomycin penetrated very slowly across the meninges, and CSF levels only slightly in excess of their MICs against *M. tuberculosis* were achieved. The penetration of the drugs into the CSF correlated poorly with differences in their partitioning between octanol/water and cyclohexane/water but could be predicted using a simple model based on their renal clearance rates and plasma protein binding. It is recommended that patients with tuberculous meningitis should be treated for at least 9 months with a combination of isoniazid, rifampin, and pyrazinamide, which may be supplemented in the first 2 months with streptomycin.—Authors' Abstract

Ellner, J. J., Hinman, A. R., Dooley, S. W., Fischl, M. A., Sepkowitz, K. A., Goldberger, M. J., Shinnick, T. M., Iseman, M. D. and Jacobs, W. R. Tuberculosis symposium—Emerging problems and promise. J. Infect. Dis. **168** (1993) 537–551.

Between 1985 and 1991, 39,000 cases of tuberculosis occurred in excess of those expected based on previous trends. Immigration from high-prevalence countries, coinfection with human immunodeficiency virus (HIV), and outbreaks in congregative facilities are most responsible for the increase. Coincident with the increase in tuberculosis, outbreaks of multidrug resistant (MDR) tuberculosis have occurred. Clinical and epidemiologic data support nosocomial transmission. MDR tuberculosis occurred late in the course of HIV infection and was refractory to treatment. Compounding the problems of rising incidence and increasing resistance was the sudden recognition of shortages of antituberculous drugs. The

problems currently posed by tuberculosis require new approaches to diagnosis and rapid sensitivity testing as well as assuring an adequate supply of licensed drugs and development of new drugs. A number of steps have been taken by (U.S.A.) governmental agencies to assure that the challenge is met.—Authors' Abstract

Guleria, I., Mukherjee, R. and Kaufmann, S. H. E. *In vivo* depletion of CD4 and CD8 T lymphocytes impairs *Mycobacterium w* vaccine-induced protection against *M. tuberculosis*. Med. Microbiol. Immunol. **182** (1993) 129–135.

In the present study we sought to determine the relative role of CD4 and CD8 T cells in *Mycobacterium w*-induced protective immunity against tuberculosis of mice by *in vivo* depletion with specific monoclonal antibodies (mAb). Mice were immunized first with *Mycobacterium w*, 4 weeks later treated with anti-CD4, anti-CD8, or a combination of both mAb and subsequently infected with *M. tuberculosis* H37Rv i.v. Numbers of colony-forming units in animals depleted of CD4 T cells, CD8 T cells, or both T-cell populations were significantly higher than those in control mice receiving irrelevant mAb or no mAb. Cytokine production by T-cell subsets was also determined by culturing the cells remaining after *in vivo* depletion in the presence or absence of mycobacterial antigens. CD8 (CD4 depleted) T cells produced lower levels of interferon-gamma than CD4 (CD8 depleted) T cells. These data suggest that both CD4 and CD8 T cells participate in resistance against tuberculosis induced by vaccination with *Mycobacterium w*.—Authors' Abstract

Heifets, L., Mor, N. and Vanderkolk, J. *Mycobacterium avium* strains resistant to clarithromycin and azithromycin. Antimicrob. Agents Chemother. **37** (1993) 2364–2370.

Mycobacterium avium strains susceptible to clarithromycin and azithromycin contain mutants resistant to these macrolides with a frequency of 1.1×10^{-10} to 1.2×10^{-6}. Crossresistance between clarithromycin and azithromycin was demonstrated with mutants selected in the laboratory as well as with resistant strains isolated from patients. The susceptibility-resistance patterns of the macrolide-resistant strains with drugs other than macrolides were the same as those of the original susceptible strains. The emergence of clarithromycin resistance in patients was a result of multiplication of the preexisting resistant mutants that survived the elimination of bacteria during the initial period of treatment and was an exclusive cause of the relapse of bacteremia.—Authors' Abstract

Heymann, S. J. Modelling the efficacy of prophylactic and curative therapies for preventing the spread of tuberculosis in Africa. Trans. R. Soc. Trop. Med. Hyg. **87** (1993) 406–411.

Concerns have been raised about whether the interaction between tuberculosis and human immunodeficiency virus (HIV) may lead worldwide to a recrudescent tuberculosis (TB) pandemic. These concerns are particularly grave in Africa which has a high prevalence of both TB and HIV. This study used a computer simulation model to examine the effect of TB-HIV interactions on TB prevalence and mortality in Africa. The model then assessed the impact of expanding treatment and chemoprophylaxis programs on TB prevalence and mortality over the next decade. In communities where 20% of the population is infected with HIV and 25% receive treatment for TB, deaths from TB would be 100% higher than in communities where none of the population is HIV-infected. In a population the size of Uganda's, during one decade there would be approximately an additional 530,000 deaths from TB. When 50% of patients with active TB receive treatment, one death will be averted for every 2.5 people who receive treatment. The prevalence of active TB could be cut by over 90% in a decade by providing effective chemoprophylaxis to 30% of individuals with inactive TB. In conclusion, TB is only one example of a preventable and treatable infectious disease which can be spread through casual contact and which, because of its higher prevalence among the HIV-positive population, may lead to a preventable increase in incidence of infection among the general population.—Author's Abstract

Initial therapy for tuberculosis in the era of multidrug resistance—recommendations of the Advisory Council for the Elimination of Tuberculosis [reprinted from MMWR **42** (1993) 1–8]. JAMA **270** (1993) 604.

These recommendations update previous CDC/American Thoracic Society (ATS) recommendations for the treatment of tuberculosis (TB) among adults and children. The most notable changes are in response to the increasing prevalence of drug-resistant TB in the United States. These recommendations include the need for a) *in vitro* drug susceptibility testing of *Mycobacterium tuberculosis* isolates from all patients and reporting of these results to the health department, b) initial four-drug regimens for the treatment of TB, and c) initial directly observed therapy for persons with TB. Adherence to these recommendations will help prevent the occurrence of more cases of drug-resistant TB, reduce the occurrence of treatment failure, and reduce the transmission of TB in the United States.

Iseman, M. D. Treatment of multidrug-resistant tuberculosis. N. Engl. J. Med. **32** (1993) 784–791.

The frequency of infections with *Mycobacterium tuberculosis* resistant to antituberculous drugs is increasing in the United States and globally. This increase is a major threat to tuberculosis treatment and control programs. To prevent this situation from worsening, initial treatment programs that entail directly observed therapy supported by effective inducements or enforcements must be used. Retreatment of patients who have multidrug-resistant tuberculosis should be carried out in programs with comprehensive microbiologic, pharmacokinetic, psychosocial, and nutritional support systems. Regimens of multiple drugs, which generally are poorly tolerated and more toxic than traditional regimens, must be administered for 18 to 36 months. Resectional surgery may be required for substantial numbers of patients. For patients with AIDS who acquire tuberculosis caused by multiply-resistant strains, the disease may prove lethal before effective therapy can be implemented. Ultraviolet irradiation systems should be used to protect health care personnel and other patients in high-risk environments. Enhanced federal, state, and local programs for prevention and control are urgently needed, and research to identify new medications and systems for their delivery is essential.—Author's Summary

Ishiyama, T., Watanabe, K., Fukuchi, K., Yajima, K., Koike, M., Tomoyasu, S. and Tsuruoka, N. The presence of CD5(LOW+) NK cells in normal controls and patients with pulmonary tuberculosis. Immunol. Lett. **37** (1993) 139–144.

CD5 antigen is present on all normal alpha/beta T cells and some B cells. Human natural killer (NK) cells do not usually express CD5 antigen, but we found a subset of CD5LOW+ (low density of CD5) NK cells in some patients with pulmonary tuberculosis. Unlike CD5 NK cells, most CD5LOW+ NK cells had HLA-DR. We observed few CD5LOW+ NK cells in the normal controls and some in the large granular lymphocyte (LGL) population purified by Percoll density centrifugation. Sorted CD5LOW+ NK populations were LGL. The CD5LOW+ NK cells had high lytic activity on K562 cells in a 4-hr chromium-51 release assay. Our results indicate that there is a previously unidentified subset of NK cells.—Authors' Abstract

Jereb, J. A., Burwen, D. R., Dooley, S. W., Haas, W. H., Crawford, J. T., Geiter, L. J., Edmond, M. B., Dowling, J. N., Shapiro, R., Pasculle, A. W., Shanahan, S. L. and Jarvis, W. R. Nosocomial outbreak of tuberculosis in a renal transplant unit—application of a new technique for restriction fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolates. J. Infect. Dis. **168** (1993) 1219–1224.

From January 1990 through February 1991, tuberculosis (TB) developed in 10 renal transplant (RT) patients at one hospital; 5 patients died. Possible nosocomial transmission was investigated. *Mycobacterium tuberculosis* isolates were compared by restriction fragment length polymorphism (RFLP) by a polymerase chain reaction

method. The source case occurred in an RT patient (source) who had posttransplant exposure to TB at another hospital. The source patient was rehospitalized on the RT unit; diagnosis of TB and thus isolation precautions were delayed. Epidemiologic and RFLP analysis showed transmission from the source to 5 RT patients and 1 human immunodeficiency virus-infected patient. *M. tuberculosis* isolates from 4 RT patients had other RFLP patterns. The median incubation period for TB in RT patients was 7.5 weeks (range, 5–11). Bronchoscopy and intubation of the source patient and inadequate ventilation on the RT unit possibly increased transmission. Early detection of TB and effective isolation are essential to prevent nosocomial transmission.—Authors' Abstract

Jonas, V., Alden, M. J., Curry, J. I., Kamisango, K., Knott, C. A., Lankford, R., Wolfe, J. M. and Moore, D. F. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J. Clin. Microbiol. **31** (1993) 2410–2416.

Seven-hundred-fifty-eight processed sputum sediments received for the diagnosis of tuberculosis or other mycobacterial infections were tested by utilizing a rRNA target amplification assay and traditional culture techniques. The results from the rRNA target amplification assay (Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test), available in 5 hr, were compared with the results from standard culture techniques held for 6 weeks. A total of 119 specimens (16%) were culture positive for *Mycobacterium tuberculosis*. Overall sensitivity, specificity, positive predictive value, and negative predictive value were 82, 99, 97, and 96%, respectively, for the Gen-Probe assay; 88, 100, 100, and 97%, respectively, for culture; and 53, 99.8, 99.6, and 91%, respectively, for fluorochrome stain. The Gen-Probe assay employs the isothermal enzymatic amplification of *M. tuberculosis* complex rRNA followed by detection of the amplicon with an acridinium ester-labeled DNA probe. This assay has the potential of reducing the time for diagnosis of tuberculosis to 1 day.—Authors' Abstract

Kamijo, R., Le, J., Shapiro, D., Havell, E. A., Huang, S., Aguet, M., Bosland, M. and Vilček, J. Mice that lack the interferon-γ receptor have profoundly altered responses to infection with bacillus Calmette-Guérin and subsequent challenge with lipopolysaccharide. J. Exp. Med. **178** (1993) 1435–1440.

Mice with a targeted disruption of the interferon-γ receptor gene (IFN-$\gamma R^{0/0}$ mice) and control wild-type mice were inoculated with the bacillus Calmette-Guérin (BCG) strain of *Mycobacterium bovis*. BCG infection was not lethal for wild-type mice whereas all IFN-$\gamma R^{0/0}$ mice died ~7–9 wk after inoculation. Histological examination at 2 and 6 wk after BCG inoculation showed that livers of IFN-$\gamma R^{0/0}$ mice had higher numbers of acid-fast bacteria than wild-type mice, especially at 6 wk. In parallel, the livers of IFN-$\gamma R^{0/0}$ mice showed a reduction in the formation of characteristic granulomas at 2 wk after inoculation. Injection of lipopolysaccharide (LPS) 2 wk after BCG inoculation was significantly less lethal for IFN-$\gamma R^{0/0}$ mice than for wild-type mice. Reduced lethality of LPS correlated with a drastically reduced production of tumor necrosis factor-α (TNF-α) in the IFN-$\gamma R^{0/0}$ mice. Interleukin 1α (IL-1α) and IL-6 levels in the serum were also significantly reduced in the IFN-$\gamma R^{0/0}$ mice after BCG infection and LPS challenge. The greatly reduced capacity of BCG-infected IFN-$\gamma R^{0/0}$ mice to produce TNF-α may be an important factor in their inability to resist BCG infection. These results show that the presence of a functional IFN-γ receptor is essential for the recovery of mice from BCG infection, and that IFN-γ is a key element in the complex process whereby BCG infection leads to the sensitization to endotoxin.—Authors' Summary

Kinger, A. K. and Tyagi, J. S. Identification and cloning of genes differentially expressed in the virulent strain of *Mycobacterium tuberculosis*. Gene **131** (1993) 113–117.

The mechanism(s) used by *Mycobacterium tuberculosis* to establish disease in the human host are not well understood. The virulent *M. tuberculosis* H37Rv strain and

its avirulent derivative *M. tuberculosis* H37Ra provide an attractive system for the identification of virulence-specific genes of the tubercle bacillus. Differentially expressed genes in the virulent strain of *M. tuberculosis* (dev genes) were identified by screening a plasmid gene bank of H37Rv with a cDNA probe that was enriched in dev transcripts by subtraction of RNAs common to H37Ra. Individual dev clones coded for RNA transcripts that were differentially expressed in H37Rv in comparison to H37Ra. In contrast, mRNAs and stable RNAs that were commonly expressed in both the strains were present in equivalent amounts. The identification and cloning of dev genes marks the first step in defining bacterial gene(s) involved in the pathogenesis of *M. tuberculosis*.—Authors' Abstract

Kirschbaum, T. M. and Gotte, R. F. C. Rifampicin resistance of Mu lysogenic strains by rpoB mutations. Biol. Chem. Hoppe Seyler **374** (1993) 657–664.

The resistance of *Escherichia coli* against the antibiotic rifampin is caused by mutations in the rpoB gene which alter the structure of the beta subunit of the DNA-dependent RNA polymerase. By insertion-mutagenesis with bacteriophage Mu rifampin-resistant mutants that were believed to have a sensitive RNA polymerase had been generated. For closer analysis of this putative rpoB-independent mechanism of resistance we cloned and sequenced the insertion sites from two of the Mu lysogens. One of them showed > 95% sequence similarity with the phn locus of *E. coli* B, and was mapped between nt 3824 and 3825 within the phnB gene. This positions the phn locus between 4397 and 4410 kb of the Kohara map of *E. coli* K-12. Since both analyzed insertion sites differ in respect to each other and to the findings of previous work we determined the rifampin sensitivity of the RNA polymerase of the Mu lysogens with partially purified enzyme. For all mutants the IC50 as a measure for sensitivity was significantly higher than for the parent strain. Sequence analysis of part of the rpoB gene (nt 1519 to 1716) revealed single point mutations in the investigated Mu lysogens. The rpoB mutation is necessary and sufficient for the observed resistance, while the prophage alone does not evoke resistance and has no synergistic effect together with the rpoB mutation. Our work supports the assumption that rifampin like other hydrophobic molecules enters the cell via simple diffusion through the outer membrane with LPS being the main barrier. The existence of a transport system for rifampin as was found for the rifamycin derivative CGP 4832 is highly unlikely. Because there is a concentration gradient of ca. 3 orders of magnitude between rifampin outside and inside the cell only subtle changes in the tolerance of the RNA polymerase are sufficient to cause a significant phenotypic resistance. The *in vitro* system used in previous studies had not been precise enough to resolve these small differences.—Authors' Abstract

Klemens, S. P., DeStefano, M. S. and Cynamon, M. H. Therapy of multidrug-resistant tuberculosis: lessons from studies from mice. Antimicrob. Agents Chemother. **37** (1993) 2344–2347.

The activities of antituberculosis agents were evaluated in a murine tuberculosis model using a drug-resistant isolate. A multidrug-resistant clinical isolate from a recent outbreak of tuberculosis in the New York State (U.S.A.) correctional system was used for infection. Approximately 10^7 viable *Mycobacterium tuberculosis* ATCC 49967 (strain CNL) organisms were given intravenously to 4-week-old, female outbred mice. Treatment was started 1 day after infection and given for 4 weeks. Spleens and lungs were homogenized, and viable cell counts were determined. Statistical analysis indicated that ethionamide, sparfloxacin, ofloxacin, capreomycin, clarithromycin, and clofazimine are active in the murine test system with this multidrug-resistant tuberculosis isolate. Sparfloxacin is the most active quinolone. Despite *in vitro* resistance, isoniazid has moderate activity. *In vitro* susceptibility data coupled with evaluation of agents against drug-resistant isolates in the murine system should provide information necessary to design clinical trials for treatment of infections with these organisms.—Authors' Abstract

Klopman, G., Wang, S. M., Jacobs, M. R., Bajaksouzian, S., Edmonds, K. and Ellner, J. J. Anti-*Mycobacterium avium* activity of quinolones—*in vitro* activities. Antimicrob. Agents Chemother. **37** (1993) 1799–1806.

The MICs of 88 quinolones against 14 selected reference and clinical strains of *Mycobacterium avium-M. intracellulare* complex were determined. Agents tested included ciprofloxacin, sparfloxacin (PD 131501), and 86 other experimental quinolones. Test strains were selected to represent various susceptibilities to ciprofloxacin and other drug-resistance profiles. MICs were determined by the microdilution method in 7HSF broth, with incubation for 14 days at 35°C. The results showed 25 of the quinolones to be active against the strains, with MICs for 90% of the strains (MIC90s) of 2 to 32 μg/ml. Ten of these compounds had activities equivalent to or greater than that of ciprofloxacin. The most active compound was PD 125354, with an MIC50 of 0.5 μg/ml and an MIC90 of 2 μg/ml; comparable values for ciprofloxacin were 4 and 8 μg/ml, respectively. The next most active compounds, with MIC90s of 4 μg/ml, were sparfloxacin (PD 131501), PD 123982, PD 135144, and PD 119421. MIC90s of PD 131575, PD 126889, PD 122642, PD 139586, and PD 143289 were 8 μg/ml. Further evaluation of the most active agents is warranted, as is assessment of structure-activity relationships of active and inactive agents to elucidate the active portions of the compounds and to lead to the development of compounds with enhanced activity.—Authors' Abstract

Leão, S. C. Tuberculosis—new strategies for the development of diagnostic tests and vaccines. Braz. J. Med. Biol. Res. **26** (1993) 827–833.

New diagnostic tests and vaccines for tuberculosis are being developed by means of a strategy based on the study of antigens exclusive to *Mycobacterium tuberculosis*. These antigens were initially identified by Western blots using sera from active pulmonary tuberculosis patients against sonic extracts from *M. tuberculosis* and *M. bovis* BCG. Several proteins present in the *M. tuberculosis* but absent in the *M. bovis* BCG sonic extracts were selected and are currently under investigation. One of these, denoted MTP40, has been extensively studied. The nucleotide sequence of the mtp40 gene has been obtained; hybridization studies have shown that this DNA fragment is exclusive to *M. tuberculosis*. Using this genomic fragment, a polymerase chain reaction (PCR)-based diagnostic test which allows the specific identification of a minimum of 10 fg of *M. tuberculosis* DNA was developed. The diagnostic assay is now being tested on uncultured clinical samples in order to determine its usefulness in routine diagnosis. Peptides synthesized from the derived sequence for the MTP40 protein and also from other *M. tuberculosis* proteins are now being studied as possible candidates for a new generation of synthetic vaccines against tuberculosis.—Author's Abstract

Leão, S. C., Lopes, J. D. and Patarroyo, M. E. Immunological and functional characterization of proteins of the *Mycobacterium tuberculosis* antigen 85 complex using synthetic peptides. J Gen. Microbiol. **139** (1993) 1543–1549.

As tuberculosis re-emerges as an important health problem worldwide, new drugs, better diagnostic tests and vaccines are being sought. In order to identify potentially useful peptides for the development of a synthetic vaccine against tuberculosis, immunological and functional studies were performed using proteins of the antigen 85 complex. Western blot (immuno-blot) analysis and a lymphoproliferaton study were used to investigate the B- and T-cell immune response of tuberculosis patients, healthy household contacts and normal controls to proteins of the *Mycobacterium tuberculosis* antigen 85 complex. Peptides derived from the 85A amino-acid sequence were synthesized and used in fibronectin-binding and in ELISAs. A peptide with the sequence CQPACRKAGCQTYKWEC bound to radiolabeled fibronectin in a time-dependent manner and was recognized by human sera in ELISA. This peptide was identified as a potential component of a synthetic vaccine against tuberculosis.—Authors' Abstract

Liipo, K. K., Kulmala, K. and Tala, E. O. J. Focusing tuberculosis contact tracing by smear grading of index cases. Am. Rev. Respir. Dis. **148** (1993) 235–236.

Several studies have confirmed that the contacts of sputum-smear-positive patients are a high-risk group. We made a prospective survey to investigate the contacts of infectious cases of pulmonary tuberculosis in order to evaluate whether different grades of sputum-smear positivity have any consequence in the emergence of new cases. The number of contacts reported by 134 index cases was 609. These included 136 (22%) who had been in close contact to the index case, 69 of them to patients heavily positive by sputum smear. Tracing of 609 contacts over 2 years revealed four (0.7%) new cases of active tuberculosis. All of them were close contacts and, moreover, all four (5.8%) belonged to the group of 69 whose index case had a heavily positive sputum smear. This was significantly more than in other contacts, $p = 0.0002$. To be productive, tracing should be limited to close contacts of heavily smear-positive patients. This seems also to be the group in which chemoprophylaxis could be cost-effectively focused.—Authors' Abstract

Maekura, R., Nakagawa, M., Nakamura, Y., Hiraga, T., Yamamura, Y., Ito, M., Ueda, E., Yano, S., He, H., Oka, S., Kashima, K. and Yano, I. Clinical evaluation of rapid serodiagnosis of pulmonary tuberculosis by ELISA with cord factor (trehalose-6,6′-dimycolate) as antigen purified from *Mycobacterium tuberculosis.* Am. Rev. Respir. Dis. **148** (1993) 997–1001.

Immunoglobulin G (IgG) antibodies against purified cord factor (trehalose-6,6′-dimycolate) prepared from *Mycobacterium tuberculosis* H37Rv were determined by enzyme-linked immunosorbent assay (ELISA), and its diagnostic usefulness was evaluated. Serum specimens from 65 patients with active pulmonary tuberculosis, 58 patients with inactive pulmonary tuberculosis, 36 patients with diseases other than tuberculosis, and 66 healthy adults were examined. Patients with active pulmonary tuberculosis showed significantly higher titers of IgG antibodies against cord factor than did other groups ($p < 0.001$). The antibody titer greater than 0.29 in absorption difference (492 to 630 nm) of 160-times diluted serum was set as positive in ELISA. For patients with active and untreated pulmonary tuberculosis, the ELISA had a sensitivity of 81% and a specificity of 96%. From these results, it was concluded that the detection of IgG antibodies against cord factor is useful for the serodiagnosis of active pulmonary tuberculosis. It was also indicated that the anticord factor antibody titers decline to the normal level as a result of antituberculosis chemotherapy.—Authors' Abstract

Mariani, F., Piccolella, E., Colizzi, V., Rappuoli, R. and Gross, R. Characterization of an IS-like element from *Mycobacterium tuberculosis.* J. Gen. Microbiol. **139** (1993) 1767–1772.

A DNA sequence, present in members of the *Mycobacterium tuberculosis* complex, has been identified and characterized. The distribution of this DNA sequence among mycobacterial species was analyzed by DNA hybridization and PCR experiments. Since the sequence was detected only in bacteria belonging to the *M. tuberculosis* complex, it may be useful for the rapid discrimination of mycobacteria. Interestingly, the sequence has some characteristics of an insertion element (IS) and codes for a hypothetical protein with significant homologies to proteins encoded by several IS elements of other organisms, namely, IS427 and IS869 from *Agrobacterium tumefaciens,* IS402 from *Pseudomonas cepacia,* Tn4811 from *Streptomyces lividans* and ISRm4 from *Rhizobium meliloti.* Together, these elements form a previously unrecognized family of transposable elements. This finding suggests the possibility of horizontal gene transfer between pathogenic mycobacteria and other organisms including gram-negative plant-pathogenic bacteria.—Authors' Abstract

Morisaki, N., Iwasaki, S., Yazawa, K., Mikami, Y. and Maeda, A. Inactivated products of rifampicin by pathogenic *Nocardia* spp.—structures of glycosylated and phosphorylated metabolites of rifampicin and 3-formylrifamycin SV. J. Antibiot. **45** (1993) 1605–1610.

Rifampin (1) was converted into four inactivated products by pathogenic *Nocardia*, RIP-1 and RIP-2 by *N. brasiliensis* and RIP-3 and RIP-4 by *N. otitidiscaviarum*. MS and NMR analysis showed the compounds to be 3-formyl-23-[*O*-(beta-D-glucopyranosyl)]rifamycin SV (2), 23-[*O*-(beta-D-glucopyranosyl)]rifampicin (3), 21-(*O*-phosphoryl)rifampin (4) and 3-formyl-21-(*O*-phosphoryl)-rifamycin SV (5), respectively.—Authors' Abstract

Orme, I. M. Immunity to mycobacteria. Curr. Opin. Immunol. **5** (1993) 497–502.

Recent progress in the field of immunity to mycobacteria has centered on T-cell subset responses and the cytokines these cells secrete. In addition, there has been steady progress in identifying and characterizing several classes of major mycobacterial proteins; included among these are the secreted/export proteins of *Mycobacterium tuberculosis*, which several laboratories now believe may represent the key protective immunity-inducing antigens of the bacillus.—Authors' Abstract

Perraut, R., Lussow, A. R., Gavoille, S., Garraud, O., Matile, H., Tougne, C., Van Embden, J., Vanderzee, R., Lambert, P. M., Gysin, J. and Delgiudice, G. Successful primate immunization with peptides conjugated to purified protein derivative or mycobacterial heat shock proteins. Clin. Exp. Immunol. **93** (1993) 382–386.

We have previously shown in mice that antibodies can be induced to synthetic malaria peptides conjugated to mycobacterial antigens, such as purified protein derivative (PPD) or heat-shock proteins (hsp), and given in the absence of adjuvants after a previous priming with bacille Calmette-Guérin (BCG). In the present study we investigated this model of immunization in the nonhuman primates, *Saimiri sciureus* monkeys. Monkeys primed with BCG subcutaneously and then immunized subcutaneously with the *Plasmodium falciparum* sporozoite (NANP)40 synthetic peptide conjugated to PPD or mycobacterial hsp of 65 or 70 kDa, in the absence of adjuvants, produced anti-peptide or anti-sporozoite IgG antibodies. Interestingly, the carrier effect of the hsp of 70kDa for the induction of anti-(NANP)40 antibodies was also observed in the absence of a previous priming with BCG. These data suggest that such a vaccination strategy may be applied to humans.—Authors' Abstract

Plikaytis, B. B., Crawford, J. T., Woodley, C. L., Butler, W. R., Eisenach, K. D., Cave, M. D. and Shinnick, T. M. Rapid, amplification-based fingerprinting of *Mycobacterium tuberculosis*. J. Gen. Microbiol. **139** (1993) 1537–1542.

Insertion element IS*6110* occurs in multiple copies throughout the *Mycobacterium tuberculosis* genome, and the variability of its insertion sites is the basis for the IS*6110* restriction fragment length polymorphism (RFLP) method for typing. We describe a novel gene amplification method to assess the variability of the location of IS*6110*. A unilateral-nested polymerase chain reaction and hybridization procedure was used to measure the variability in the distances between IS*6110* elements and copies of a major polymorphic tandem repeat sequence of *M. tuberculosis*. The pattern of amplicons produced could be used to cluster epidemiologically related strains of *M. tuberculosis* into groups which correlated with the groups formed using IS*6110*-RFLP typing. Reliable patterns can be generated directly from sputum specimens as well as from *M. tuberculosis* cultures. We designated the novel method as IS*6110*-ampliprinting.—Authors' Abstract

Prinzis, S., Chatterjee, D. and Brennan, P. J. Structure and antigenicity of lipoarabinomannan from *Mycobacterium bovis* BCG. J. Gen. Microbiol. **139** (1993) 2649–2658.

Lipoarabinomannan (LAM), a major lipoglycan of the mycobacterial cell envelope, was previously recognized as existing in two major forms: LAM with arabinofuranosyl (Ara*f*)-containing termini (AraLAM) and a mannose-capped version (ManLAM) in which the majority of these termini are modified by additional mannose residues. Since ManLAM was first recognized in the virulent (Erdman) strain of *Mycobacterium tuberculosis* and the noncapped version in

a rapidly growing, attenuated, H37Ra strain, it was thought that mannose capping may be a key factor in virulence. In the present study, LAM from *M. bovis* BCG was isolated and the nonreducing termini sequenced through differential *O*-alkylation, partial depolymerization and gas chromatography-mass spectrometric analyses of fragments. LAM from *M. bovis* BCG contains a short mannan backbone, highly branched arabinofuranosyl-containing side chains and several mannosyl residues capping the nonreducing termini of these side chains. Thus, LAM from *M. bovis* BCG is of the ManLAM type, showing no major structural differences at the nonreducing ends from the *M. tuberculosis* Erdman product. This observation led us to examine the earlier strain and to conclude that it showed little resemblance to conventional strains of *M. tuberculosis*. Thus, the absence of mannose caps may be more a feature of rapid growth than of avirulence. These results demonstrate that the relationship between mannose capping and disease induction is not a simple one. However, use of a panel of LAM-specific monoclonal antibodies showed antigenic differences between the BCG and the Erdman products, suggesting the presence of features specific to the different strains and pointing to LAM as a molecule within which further species and strain variations reside.—Authors' Abstract

Recommendations on prophylaxis and therapy for disseminated *Mycobacterium avium* complex for adults and adolescents infected with human immunodeficiency virus. MMWR **42** (1993) 17–20.

Mycobacterium avium complex (MAC) causes disseminated disease in up to 40% of patients with advanced human immunodeficiency virus (HIV) disease in the United States. A U.S. Public Health Service Task Force convened to address the prophylaxis and therapy of MAC recommends that patients with HIV infection and < 100 CD4+ T-lymphocytes/μl be administered prophylaxis against MAC. The recommended regimen is rifabutin, 300 mg by mouth daily, for the patient's lifetime. If disseminated MAC develops, a treatment regimen containing clarithromycin or azithromycin and at least one other agent is recommended. Diagnosis, therapy, and prophylaxis for HIV-infected children follow similar guidelines.—Summary

Saceanu, C. A., Pfeiffer, N. C. and McLean, T. Evaluation of sputum smears concentrated by cytocentrifugation for detection of acid-fast bacilli. J. Clin Microbiol. **31** (1993) 2371–2374.

Early identification and isolation of tuberculosis patients is of utmost importance to minimize the risk of further epidemic spread of the disease. The traditional concentrated acid-fast smears are not very reliable tools for the presumptive diagnosis of tuberculosis. Acid-fast bacillus (AFB) smears from 120 patient specimens and 80 simulated AFB samples were processed according to standard laboratory procedures and by cytocentrifugation (Cyto-Tek, Ames Division, Miles Laboratories, Inc., Elkhart, Indiana, U.S.A.). Prior to dispensing of samples into the Cyto-Tek chambers, specimens were liquefied and decontaminated by mixture with an equal volume of 5% sodium hypochlorite (household bleach). Culture and smear results were correlated. Of 120 patient specimens, 43 were culture- and smear-negative by both methods. Ten specimens were overgrown with mold and bacteria, but 2 of them had positive AFB smears by cytocentrifugation only. There were 67 positive AFB cultures, with 67 positive cytocentrifuge smears and 34 positive smears by the conventional technique. Of the 80 simulated positive AFB samples, all grew mycobacteria on culture. Smears from the 10^5–10^3-CFU/ml specimens were positive by both methods. The simulated samples with 10^2 CFU/ml yielded smears positive only by cytocentrifugation. The Cyto-Tek AFB smears had a greater correlation with positive culture than did the smears from concentrated specimens. The sensitivity, efficiency, and rapidity of the Cyto-Tek AFB smear technique resulted in increased detection of mycobacteria in clinical specimens. The simplicity and safety of this method will enable qualified mycobacteriology technologists to rapidly and accurately perform sputum smears for AFB at clinics, emergency rooms, and field sites, as well as in the traditional laboratory setting.—Authors' Abstract

Sippola, A. A., Gillespie, S. L., Lewis, J. A. and Daniel, T. M. *Mycobacterium avium* antigenuria in patients with AIDS and disseminated *M. avium* disease. J. Infect. Dis. **168** (1993) 466–468.

A 22,500-Da antigen apparently specific to *Mycobacterium avium, M. intracellulare,* and *M. scrofulaceum* has been identified by Western immunoblotting. By use of a dot immunoassay, this antigen was found in the urine of 64% of patients with AIDS and disseminated *M. avium* disease. It was not found in the urine of healthy control subjects. Detection of antigenuria might provide the basis for rapid diagnosis of *M. avium* disease in patients with AIDS.—Authors' Abstract

Stoeckle, M. Y., Guan, L., Riegler, N., Weitzman, I., Kreiswirth, B., Kornblum, J., Laraque, F. and Riley, L. W. Catalase-peroxidase gene sequences in isoniazid-sensitive and isoniazid-resistant strains of *Mycobacterium tuberculosis* from New York City. J. Infect. Dis. **168** (1993) 1063–1065.

Isoniazid resistance in *Mycobacterium tuberculosis* is associated with lack of catalase-peroxidase activity. A recent study showed that some isoniazid-resistant *M. tuberculosis* strains have a complete deletion of the gene (*katG*) encoding this enzyme. To examine what proportion of clinical isolates of *M. tuberculosis* have *katG* deletion, *katG* sequences in 80 randomly selected isolates from New York City were analyzed. Polymerase chain reaction was used to amplify a 282-bp segment of *M. tuberculosis katG* and showed that 35 (90%) of 39 isoniazid-sensitive and 31 (76%) of 41 isoniazid-resistant strains contained *katG* sequences ($p > 0.1$). Ten multidrug and high-level isoniazid-resistant strains with identical restriction fragment length polymorphism patterns were also analyzed. All were found to have *katG* sequences. These findings suggest that mechanisms other than complete deletion of *katG* are involved in isoniazid resistance among most clinical isolates of *M. tuberculosis* from New York City.—Authors' Abstract

Thomas, L., Ducros, B., Secchi, T., Balme, B. and Moulin, G. Successful treatment of adults Langerhans' cell histiocytosis with thalidomide—report of 2 cases and literature review. Arch. Dermatol. **129** (1993) 1261–1264.

Our cases represent two new observations of systemic Langerhans' cell histiocytosis with cutaneous manifestations in adults. In both cases, the diagnosis was definitely established according to the criteria of the Histiocyte Society. The specific interest in these two cases is the dramatic improvement of the cutaneous manifestations of the disease with thalidomide therapy within a short period of time and without any clinical or electrophysiologic side effects.—From the Authors' Comment

Vaneechoutte, M., Debeenhouwer, H., Claeys, G., Vershraegen, G., Derouck, A., Paepe, N., Elaichouni, A. and Portaels, F. Identification of *Mycobacterium* species by using amplified ribosomal DNA restriction analysis. J. Clin. Microbiol. **31** (1993) 2061–2065.

A rapid procedure for the identification of cultured *Mycobacterium* isolates, based on the combination of enzymatic amplification and restriction analysis, is described. The 16S rRNA genes (rDNA) of 99 strains belonging to 18 different species of the genus *Mycobacterium* were enzymatically amplified. Amplified rDNA restriction analysis with the enzymes CfoI, MboI, and RsaI was carried out. The combination of the amplified rDNA restriction analysis patterns obtained after restriction with CfoI and MboI enabled differentiation between *Mycobacterium asiaticum* (number of strains = 4), *M. avium* (N = 22), *M. chelonae* (N = 5), *M. flavescens* (N = 1), *M. fortuitum* (N = 6), *M. gordonae* (N = 6), *M. intracellulare* (N = 13), *M. marinum* (N = 7), *M. nonchromogenicum* (N = 1), *M. simiae* (N = 5), *M. terrae* (N = 5), the *M. tuberculosis* complex (N = 11), and 2 of 4 strains of *M. xenopi.* Further restriction with RsaI was necessary to differentiate between the species of *M. kansasii* (N = 5), *M. scrofulaceum* (N = 4), and the 2 other *M. xenopi* strains. The *M. avium-M. intracellulare* complex was characterized by a specific MboI pattern, and *M. avium* and *M. intracellulare* strains could further be differentiated by restriction with CfoI. The whole procedure,

including sample preparation prior to the polymerase chain reaction, can be carried out within 8 hr, starting from a pure culture.—Authors' Abstract

Vordermeier, H. M., Harris, D. P., Lathigra, R., Roman, E., Moreno, C. and Ivanyi, J. Recognition of peptide epitopes of the 16,000-MW antigen of *Mycobacterium tuberculosis* by murine T cells. Immunology **80** (1993) 6–12.

The T-cell repertoire to a prominent immunogen of *Mycobacterium tuberculosis* has been investigated on the assumption that differences in epitope specificity could influence the protective and pathogenic host reactions. Proliferative responses of lymph node and spleen cells to overlapping peptides, spanning the entire sequence of the 16,000-MW protein antigen were analyzed in C57BL/10 and B10.BR mice. Following foot pad priming and *in vitro* challenge with homologous peptide, 12 out of the 14 peptides tested were found to be immunogenic. However, only two peptides of residues 31–40 and 71–91 simulated strong proliferative responses of T cells from mice which had been presensitized with either killed or live *M. tuberculosis* organisms; another three peptides were only weakly stimulatory. These epitopes have been immunodominant in both H-2b and H-2k mouse strains, indicating the genetically permissive nature of their recognition. Furthermore, both major immunodominant epitopes were found to be species specific for the *M. tuberculosis* complex and therefore potentially suitable for the early diagnosis of tuberculous infection.—Authors' Abstract

Wallace, R. J., Tanner, D., Brennan, P. J. and Brown, B. A. Clinical trial of clarithromycin for cutaneous (disseminated) infection due to *Mycobacterium chelonae.* Ann. Intern. Med. **119** (1993) 482–486.

The objective was to determine if clarithromycin monotherapy is safe and effective in treating cutaneous disease (especially disseminated disease) due to *Mycobacterium chelonae* (formerly *M. chelonae* subspecies *chelonae*). The patients were culture-positive patients whose *M. chelonae* came from a cutaneous source and whose isolate was submitted to a single referral laboratory for susceptibility testing. Clarithromycin, 500 mg twice a day by mouth for 6 months was given. No attempt was made to alter use of immunosuppressive drugs. The main outcome measures were acid-fast bacilli smears and cultures of skin lesions during and after treatment, with monitoring of clinical response, side effects, and development of new lesions.

Fourteen patients (10 with disseminated disease) were enrolled in the study and completed at least 3 months of therapy. Underlying diseases included rheumatoid arthritis, other autoimmune disorders, and organ transplantation. All were taking corticosteroids (93%) or cyclophosphamide (7%). All patients had an excellent response to therapy, with only mild side effects from the drug. Two patients died of other diseases after improving clinically but while still taking medication. One noncompliant patient who prematurely discontinued therapy after 3.5 months relapsed 1 month later with an isolate resistant to clarithromycin. The remaining 11 patients have all completed therapy given for a mean of 6.8 months (range, 4.5 to 9 months). Therapy has been discontinued for 9 of the 11 patients for at least 6 months (mean, 7.1 months; range, 6 to 12 months), with no evidence of relapse. No remaining patient had positive acid-fast bacilli smears or cultures of skin lesions after 1 month of therapy. Clarithromycin may be the drug of choice for cutaneous (disseminated) disease due to *M. chelonae,* although more patients with long-term clinical follow-up need to be studied.—Authors' Abstract

White, P. C. L., Brown, J. A. and Harris, S. Badgers (*Meles-meles*), cattle and bovine tuberculosis (*Mycobacterium bovis*)—a hypothesis to explain the influence of habitat on the risk of disease transmission in southwest England. Proc. R. Soc. London [Biol.] **253** (1993) 277–284.

Badgers are believed to be responsible for a high proportion of the cases of bovine tuberculosis in cattle in southwest England where, despite the onset of badger control operations in 1975, comparatively high numbers of cattle continue to fail the tuberculin test. To determine why the disease remains a problem in these areas, data on

badger densities and patterns of land use were examined. Areas subject to repeated badger control operations had greater landscape heterogeneity and a higher density of linear habitat features. These habitat features were not related to badger density, measured as the mean number of social groups per square kilometer. Environmental contamination by infected badger urine is thought to be the main mode for the transmission of bovine tuberculosis. Field studies in an area with tuberculosis in both badgers and cattle showed that badgers may urinate on pasture after crossing through a linear feature, and that the number of these crossing-point urinations increases with the number of linear features crossed. The hypothesis is presented that these crossing-point urinations are a major source of bovine tuberculosis infection in cattle, and that areas with increased numbers of linear features have greater levels of contamination of pasture with badger urine and hence increased opportunities for disease transmission.—Authors' Abstract

Wolucka, B. A., McNeil, M. R., Kalbe, L., Cocito, C. and Brennan, P. J. Isolation and characterization of a novel glucuronosyl diacylglycerol from *Mycobacterium smegmatis*. Biochim. Biophys. Acta **1170** (1993) 131–136.

A glucuronic acid-containing diacylglycerol was isolated from exponentially growing *Mycobacterium smegmatis*. Structural analysis of the purified glycolipid, performed by gas chromatography-mass spectrometry, fast atom bombardment-mass spectrometry, and high resolution proton NMR, indicated the structure 3-(*O*-alpha-D-glucuronopyranosyl)-1,2-diacyl-sn-glycerol. Two forms of the glycolipid were observed differing in fatty acid composition. Both molecular species contained a hexadecanoic acid residue; whereas the second acyl group was either tuberculostearic acid (10-methylstearic acid) or octadecenoic acid. The inherent antigenicity of the glycolipid was shown by its ability to bind to anti-*Mycobacterium avium* (serovar 26) and anti-*M. tuberculosis* sera by Western blot-type thin-layer chromatography. This is the second instance of the isolation of a glycosyl diacylglycerol from members of the *Mycobacterium* genus, further confirming its close relationship to gram-positive bacteria.—Authors' Abstract

Yang, M., Ross, B. C. and Dwyer, B. Identification of an insertion sequence-like element in a subspecies of *Mycobacterium kansasii*. J. Clin. Microbiol. **31** (1993) 2074–2079.

Analysis of a genomic DNA clone library of a strain from the genetic subspecies of *Mycobacterium kansasii* determined the existence of a repetitive insertion sequence-like element. The element is 947 bp long and is present in a minimum of 1 to 11 copies per genome. Similar to insertion sequences, it contains a 3-bp (TAG) direct repeat at its extremities and a transcription promoter-like sequence. In addition, for one of the clones sequenced, a potential cointegrate formation, a characteristic frequently observed with insertion sequences, was revealed. This insertion sequence does not contain short inverted repeats near the ends or a large open reading frame to code for a transposase enzyme. Its host range is restricted to a previously described genetic subspecies of *M. kansasii* and is not present in typical *M. kansasii* or other mycobacterial species. When used as a probe for Southern blot hybridization, significant heterogeneity between different isolates of the *M. kansasii* subspecies was observed. This repeated element will be useful in further studies on the characterization, diagnosis, and epidemiology of *M. kansasii*.—Authors' Abstract

Yates, M. D., Pozniak, A. and Grange, J. M. Isolation of mycobacteria from patients seropositive for the human immunodeficiency virus (HIV) in southeast England, 1984–92. Thorax **48** (1993) 990–995.

Tuberculosis and other mycobacterial infections are well-recognized complications of HIV infection and surveillance is thus required. All mycobacteria isolated from HIV-positive subjects and referred to the Public Health Laboratory Service South East Regional Tuberculosis Centre (SERTC) from the first such case in 1984 until the end of 1992 were reviewed.

A total of 803 mycobacteria isolated from 727 HIV-positive subjects were referred to

the SERTC during the study period. A single species was isolated from 660 patients: 150 members of the tuberculosis complex (146 *M. tuberculosis,* 2 *M. bovis,* and 2 *M. africanum*), 356 *M. avium-intracellulare* (MAI), and 154 other environmental mycobacteria. More than one mycobacterium was isolated from 67 patients. In 12 cases *M. tuberculosis* and MAI were isolated from the same patient, almost always in that sequence, with an interval of 8–41 months between isolations. Most of the 407 isolates of MAI (74%) were considered to be clinically significant and often caused disseminated disease. In other cases single isolates of MAI were obtained from sputum or feces, and occasionally such isolates preceded disseminated disease by several months. Only 33 (14%) of the 229 isolates of environmental mycobacteria other than MAI were considered clinically significant.

HIV-related mycobacterial disease is increasing in incidence in southeast England. Further studies are required to determine the significance of single isolates of MAI and other environmental mycobacteria as a guide to the need for preventive chemotherapy or immunotherapy.—Authors' Abstract