CURRENT LITERATURE

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

General and Historical

Nunzi, E. and Fiallo, P. Leprosy: the dichotomous disease. Eur. J. Dermatol. **5** (1995) 649–652.

Leprosy is an infectious disease, the development and clinical progression of which depend on the host immunological behavior. In this review we describe the general features of leprosy and review the current knowledge on immunology. In particular, this article focuses on the features of the host-parasite interaction and the manner of adaptation of *Mycobacterium leprae* to the host environment, an understanding of which provides an insight into the pathogenesis of leprosy.—Authors' Abstract

Sun, S., *et al.* [Integrated control of both TB and leprosy.] China Lepr. J. **11** (1995) 189–191. (in Chinese)

In Huangdao District, Qingdao City, institutions for both tuberculosis and leprosy control have been integrated into a chronic diseases control station in 1985, being responsible for control of TB and leprosy. In this District, there is a population of 116,000, accumulative 87 leprosy patients of which 61 were cured, and TB detection rate of 37.9/100,000. In the station there are four or five medical workers. Their work is well harmonious and was rid of isolated situation of leprosy control.—Authors' English Abstract

Chemotherapy

Aurora, M., Hortaleza, R., Salta-Ramos, G., Barcelona-Tan, J. and Abad-Venida, M. L. Dapsone syndrome in a Filipino man. Lepr. Rev. **66** (1995) 307–313.

A case of dapsone syndrome occurring in a Filipino man under treatment for multibacillary (MB) leprosy is described. The patient manifested progressive fever, erythroderma and jaundice 4 weeks after initiation of multidrug therapy (MDT) with rifampin, clofazimine and dapsone. The clinical symptoms conformed well to the dapsone syndrome first described in the 1950s, and this report proves that the syndrome does still exist. There was recovery after dapsone was omitted and therapy with systemic corticosteroids was started.

In view of this potentially fatal hypersensitivity reaction, this case report emphasizes the need for caution when initiating MDT or dapsone therapy. It is also suggested that any patient on MDT or dapsone needs to be referred immediately to a dermatologist or internist if the patient develops a skin rash during the first 2 months of treatment.—Authors' Summary

Backman, J. T., Olkkola, K. T. and Neuvonen, P. J. Rifampin drastically reduces plasma concentrations and effects of oral midazolam. Clin. Pharmacol. Ther. **59** (1996) 7–13.

Background: Midazolam is a short-acting benzodiazepine that is metabolized by CYP3A enzymes. Rifampin is a potent enzyme inducer that may seriously interact with some substrates of CYP3A4.

Methods: The possible interaction between rifampin and midazolam was investigated in a double-blind, randomized crossover study of two phases. Rifampin (600 mg once daily) or placebo was administered to 10 healthy subjects for 5 days. On the sixth day, the subjects were given 15 mg

oral midazolam. Plasma samples were collected for determination of midazolam, and pharmacodynamic effects were measured for 10 hr.

Results: Rifampin pretreatment decreased the area under the plasma midazolam concentration-time curve by 96% (i.e., from 10.2 ± 0.8 to 0.42 ± 0.05 μg·min/ml [mean ± SEM; $p < 0.001$]) and the maximum concentration by 94% (i.e., from 55 ± 4 to 3.5 ± 0.7 ng/ml [$p < 0.001$]). The elimination half-life of midazolam was decreased from 3.1 ± 0.2 to 1.3 ± 0.2 hr by rifampin ($p < 0.001$). During the rifampin phase, the pharmacodynamic effects of midazolam were markedly smaller than the effects during the placebo phase in all the tests (e.g., the Digit Symbol Substitution Test; $p < 0.001$).

Conclusions: The observed substantial decrease in plasma concentrations and effects of midazolam most likely results from induction of CYP3A4 by rifampin in both the gut wall and the liver. Orally administered midazolam is ineffective during rifampin treatment.—Authors' Abstract

Chambers, H. F., Moreau, D., Yajko, D., Miick, C., Wagner, C., Hackbarth, C., Kocagoz, S., Rosenberg, E., Hadley, W. K. and Nikaido, H. Can penicillins and other beta-lactam antibiotics be used to treat tuberculosis? Anitmicrob. Agents Chemother. **39** (1995) 2620–2624.

An increase in the number of tuberculosis cases caused by multiple-drug-resistant strains of *Mycobacterium tuberculosis* has stimulated search for new antituberculous agents. Beta-lactam antibiotics, traditionally regarded as ineffective against tuberculosis, merit consideration. Four major penicillin-binding proteins (PBPs) with approximate molecular sizes of 94, 82, 52, and 37 kDa were detected by fluorography of [H-3]penicillin-radiolabeled membrane proteins prepared from *M. tuberculosis* H37Ra. The presence of membrane-associated beta-lactamase precluded the use of membranes for assaying the binding affinities of beta-lactam antibiotics. Therefore, ampicillin affinity chromatography was used to purify these four PBPs from crude membranes in order to assay the binding affinities of beta-lactam antibiotics. Ampicillin, amoxicillin, and imipenem, betalactam antibiotics previously reported to be active *in vitro* against *M. tuberculosis*, bound to *M. tuberculosis* PBPs at therapeutically achievable concentrations. Binding of the 94-, 82-, and 52-kDa PBPs, but not the 37-kDa PBP, was associated with antibacterial activity, suggesting that these PBPs are the critical targets. Studies of mycobacterial cell-wall permeability, which was assayed with a panel of reference cephalosporins and penicillins with different charge positivities, indicated that the rate of penetration of beta-lactam antibiotics to the target PBPs could not account for resistance. Resistance could be reversed with the beta-lactamase inhibitors clavulanate or sulbactam or could be circumvented by the use of a beta-lactamase-stable drug, imipenem, indicating that mycobacterial beta-lactamase, probably in conjunction with slow penetration, is a major determinant of *M. tuberculosis* resistance to beta-lactamase antibiotics. These findings confirm *in vitro* data that *M. tuberculosis* is susceptible to some beta-lactam antibiotics. Further evaluation of these drugs for the treatment of tuberculosis in animal models and in clinical trials is warranted.—Authors' Abstract

Coleman, M. D. Dapsone toxicity: some current perspectives. Gen. Pharmacol. **26** (1995) 1461–1467.

Dapsone is a potent antiinflammatory and antiparasitic compound, which is metabolized by cytochrome P-450 to hydroxylamines, which in turn cause methemoglobinemia and hemolysis. However, during the process of methemoglobin formation, erythrocytes are capable of detoxifying the hydroxylamine to the parent drug, which may either reach the tissues to exert a therapeutic effect or return to the liver and be re-oxidized in a form of systemic cycling. This glutathione-dependent effect, combined with the un-ionized state of the drug at physiological pH, may contribute to its efficacy. Paradoxically other aspects of the glutathione-dependent cycling of the hydroxylamine metabolite may contribute to the major adverse reaction of the drug, agranulocytosis. Erythrocytes exposed to the metabolite and repeatedly washed may still release the hydroxylamine in sufficient con-

centration to kill mononuclear leukocytes *in vitro*. Thus, erythrocytes may be a conduit for the hydroxylamine to reach the bone marrow to covalently bind to granulocyte precursors, which may trigger an immune response in certain individuals and may lead to the potentially fatal eradication of granulocytes from the circulation. Attempts to increase patient tolerance to dapsone have been most successful using a metabolic inhibitor to reduce hepatic oxidation of the drug to the hydroxylamine. Methemoglobin formation in the presence of cimetidine was maintained at 30% below control levels for almost 3 mos, and patients reported side effects such as headache and lethargy were significantly reduced. Since clinical application of new and safer dapsone analogs is years away, the use of cimetidine provides an immediate route to increasing patient compliance during dapsone therapy, especially in those maintained on dapsone dosages in excess of 200 mg/day.—Author's Abstract

Dan, Z., *et al.* [Analysis of 452 cases of relapsed leprosy.] China Lepr. J. **11** (1995) 185–187. (in Chinese)

From 1963 to 1992 in Hunan Province there have been 11, 401 people cured of leprosy with DDS monotherapy or DDS plus RMP, of which 452 have relapsed, accounting for 3.9%. The period from cured to relapsed was 9.7 (1 to 28) years for MB and 6.9 (1 to 19) years for PB on the average, respectively, having no relation to the duration of taking the treatment. In the same time, 8977 new cases were detected and the proportion between the relapsed and new patients was 1 : 19.9. The causes which led to relapse mostly were not clear, but in some cases it might have certain things to do with undernutrition, overfatigue, excessive drinking, mental stress and pregnancy etc.—Authors' English Abstract

Dhople, A. M. and Ibanez, M. A. *In vivo* activities of novel benzoxazinorifamycins against *Mycobacterium leprae*. Indian J. Lepr. **67** (1995) 375–382.

Among the four newly synthesized benzoxazinorifamycin derivatives, KRM-1648 and KRM-2312 completely inhibited the multiplication of rifampin-sensitive as well as rifampin-resistant strains of *M. leprae* in the foot pads of mice. Both were found to be more potent than rifampin and were bactericidal. In combination with ofloxacin, another potent bactericidal drug against *M. leprae*, both KRM-1648 and KRM-2312 exhibited synergism. Thus, a combination of one of these benzoxazinorifamycin derivatives and ofloxacin in multidrug regimens is worth evaluating in clinical trials.—Authors' Abstract

Gao, G.-W. [DDS syndrome when taking MDT.] China Lepr. J. **11** (1995) 200–201. (in Chinese)

While using DDS monotherapy in the 1960s to 1986, there was no case of DDS syndrome to be seen among 1357 patients with leprosy, but out of 24 new cases who have taken WHO's MDT in 1986 to 1994 three patients have suffered from DDS syndrome, making up 12.5%. The author discusses its possible causes.—Author's English Abstract

Gill, H. J., Tingle, M. D. and Park, B. K. N-Hydroxylation of dapsone by multiple enzymes of cytochrome P450: implications for inhibition of haemotoxicity. Br. J. Clin. Pharmacol. **40** (1995) 531–538.

The adverse reactions associated with the administration of dapsone are believed to be caused by metabolism to its hydroxylamine. Previous reports suggest that CYP3A4 is responsible for this biotransformation. Data presented in this paper illustrate the involvement of more than one cytochrome P450 enzyme in dapsone hydroxylamine formation using human liver microsomes. Eadie-Hofstee plots demonstrated bi-phasic kinetics in several livers. No correlation could be established between hydroxylamine formation and CYP3A concentrations in six human livers ($r = -0.47$; $p = 0.34$). Studies with low molecular weight inhibitors illustrate the importance of CYP2C9 and CYP3A in dapsone N-hydroxylation.

Differential sensitivity of dapsone N-hydroxylation to selective CYP inhibitors indicated that the contribution of individual CYP enzymes varies between livers. Selective inhibition ranged from 6.8 to 44.4% by 5 μM ketoconazole, and from 24.0% to 68.4% by 100 μM sulfaphenazole. The ex-

tent of inhibition, by either ketoconazole or sulfaphenazole was dependent on the CYP3A content of the liver. The levels of expression of these cytochrome P450 enzymes may be an important determinant of individual susceptibility to the toxic effects of dapsone, and may influence the ability of an enzyme inhibitor to block dapsone toxicity *in vivo*. Because of the inability to produce complete inhibition, selective CYP inhibitors are unlikely to offer any clinical advantage over cimetidine in decreasing dapsone hydroxylamine formation *in vivo*.—Authors' Abstract

Jian, D., *et al.* [Monitoring after short-term MDT in leprosy.] China Lepr. J. **11** (1995) 181–184. (in Chinese)

For 1 to 8 years 656 persons who had had MB leprosy and completed WHO/MDT have been followed up. Significant improvement rate was 99.39%. When monitoring time reached 5 years, The BI changed to negative in 535 persons (81.6%), particularly in those who had BI below 3.0 before MDT. In the period of treatment and monitoring, lepra reaction occurred in 140 persons (21.34%), including type 1 in 47, type 2 in 61 and neuritis in 32. One person's leprosy has relapsed in the fifth year of monitoring so the authors suggest that the monitoring duration must be lengthened.—Authors' English Abstract

Mitra, A. K., Thummel, K. E., Kalhorn, T. F., Kharasch, E. D., Unadkat, J. D. and Slattery, J. T. Metabolism of dapsone to its hydroxylamine by CYP2E1 *in vitro* and *in vivo*. Clin. Pharmacol. Therapeut. **58** (1995) 556–566.

Dapsone toxicity is putatively initiated by formation of a hydroxylamine metabolite by cytochromes P450. In human liver microsomes, the kinetics of P450-catalyzed N-oxidation of dapsone were biphasic, with the Michaelis-Menten constants of 0.14 ± 0.05 and 0.004 ± 0.003 mmol/L and the respective maximum velocities of 1.3 ± 0.1 and 0.13 ± 0.04 nmol/mg protein/min (mean ± SEM). Troleandomycin (40 μmol/L) inhibited hydroxylamine formation at 100 mu mol/L dapsone by 50%; diethyldithiocarbamate (150 μmol/L) and tolbutamide (400 μmol/L) inhibited at 5 μmol/L dapsone by 50% and 20%, respectively, suggesting that the low-affinity isozyme is CYP3A4 and the high-affinity isozymes are 2E1 and 2C. Disulfiram, 500 mg, 18 hr before a 100-mg oral dose of dapsone in healthy volunteers, diminished area under the hydroxylamine plasma concentration-time curve by 65%, apparent formation clearance of the hydroxylamine by 71%, and clearance of dapsone by 26%. Disulfiram produced a 78% lower concentration of methemoglobin 8 hr after dapsone.—Authors' Abstract

Nash, K. A. and Inderlied, C. B. Genetic basis of macrolide resistance in *Mycobacterium avium* isolated from patients with disseminated disease. Antimicrob. Agents Chemother. **39** (1995) 2625–2630.

Clarithromycin (CLM) and azithromycin (AZM) are important agents in the treatment of disseminated *Mycobacterium avium* complex disease; however, monotherapy with these macrolides often leads to clinically significant resistance. The underlying resistance mechanism was investigated by comparing 23S rRNA gene sequences in the domain V region of 10 CLM-susceptible *M. avium* strains and 8 CLM-resistant strains. Four of the CLM-resistant strains were derived from CLM-susceptible strains included in this study. The only differences in the domain V sequences associated with CLM resistance were at position 2274 of the complete *M. avium* 23S rRNA gene (GenBank accession no. X74494). All the CLM-susceptible strains had an A residue at this site; whereas 7 of the 8 CLM-resistant strains had either a C, G, or T. Four of these seven CLM-resistant strains emerged during monotherapy with CLM and two emerged during AZM monotherapy, showing that resistance selected by either macrolide was associated with mutation of the 23S rRNA gene. Thermodynamic analysis of secondary rRNA structure suggests that the observed mutations cause an alteration in free energy associated with rRNA folding, which may result in a localized conformation change in assembled ribosomes. Such a shift may be important in the resistance of ribosomes to the effects of macrolides. This study, therefore, establishes a link between mutations within the 23S rRNA gene and clinically significant macro-

lide resistance in *M. avium* and also identifies a possible molecular mechanism of resistance at the level of the ribosome.—Authors' Abstract

O'Connor, R., O'Sullivan, J. F. and O'Kennedy, R. The pharmacology, metabolism and chemistry of clofazimine. Drug Metab. Rev. **27** (1995) 591–614.

Although much still remains to be understood about the chemical and pharmacological properties of clofazimine and other phenazine agents, recent advances have provided a clearer picture of how and why these agents work. They also provide strong evidence that more useful compounds in this class can be produced, and that existing agents may be more broadly useful in a variety of disease states than was initially anticipated.—Authors' Conclusion

Peat, M., Brolin, L., Ganapati, R., McDougall, A. C., Revankar, C. R. and Watson, J. W. An evaluation of the contribution of the Swedish International Development Authority (SIDA) to leprosy control in India based on the implementation of multiple drug therapy (MDT) 1981–1993. Indian J. Lepr. **67** (1995) 447–465.

The Swedish International Development Authority (SIDA) first supported the National Leprosy Control Programme in India in 1978. In 1981/82 priority was given to the implementation of multiple drug therapy (MDT), starting in two high-endemic districts, and gradually extending to a total of 19 districts in the years by 1993. SIDA then decided to undertake a detailed evaluation of its 12-year contribution and this was carried out by an international team between November 1993 and April 1994.

In terms of epidemiological and public health impact, the main results were impressive and clear-cut; 837, 519 cases (old and newly arising) were successfully treated, with few complications and a low rate of relapse. The voluntary reporting rate had improved significantly. Data relating to new case detection, child and disability rates were, however, less clear and difficult to interpret. Deficiencies were also identified in the areas of health education, community participation, gender issues, disability prevention and management, rehabilitation, operational research and assessment of cost-effectiveness. These problems should not, however, detract from the contribution of SIDA, from 1981 onward, in establishing the implementation of MDT in two "pilot" districts at an early and important stage in the history of the MDT program in India. SIDA also made significant contributions in other areas, namely pre-MDT "screening" of registers in 45 endemic districts in 1990–1993, appointment of consultant leprologists at district level, group education activities, annual meetings of voluntary agencies and the developent of a monitoring and information system, with computer facilities, at national level.

This paper describes the design and methodology, main findings and conclusions of the evaluation, based on the final report and the appendices submitted to SIDA in Stockholm in April 1994.—Authors' Abstract

Shen, L. [Relapse of leprosy for 1989–1994 in Zhejiang.] China Lepr. J. **11** (1995) 192. (in Chinese)

Up to 1994, out of 11, 830 cases of leprosy cured with DDS monothrapy in Zhejiang Province, 283 cases have relapsed, making up 2.39%. In the period of 1989 to 1994 out of 71 cases of relapsed leprosy 37 were LL, 1 BL, 6 BB, 3 BT and 24 TT originally. But in 750 persons who have taken MDT for 3 months in order to prevent relapse after DDS monothrapy there were only 4 relapsed ones, accounting for 0.53%. Most of the relapse (53.5%) occurred 2 to 10 years after cured and the rest over 10 years. The author suggests that monitoring duration should be elongated.—Author's English Abstract

Sivaprasad, N., Snehalatha, S., Lobo, D., Aschhoff, M. and Job, C. K. Viability of *Mycobacterium leprae* in lepromatous patients after five years of dapsone monotherapy supplemented with two years of multidrug therapy. Indian J. Lepr. **67** (1995) 427–433.

Eleven lepromatous (LL) leprosy patients with a bacterial index (BI) of $\geq 3+$ who had undergone 2 years of multidrug therapy (MDT) and yet had positive skin smears at the end of treatment were chosen for this study. Biopsies from the skin and lymph

nodes were histopathologically evaluated for the presence of granulomas and *M. leprae*. *M. leprae* isolated from the skin and lymph nodes were inoculated into the foot pads of normal mice to test their viability.

On histopathological examination of the biopsy specimens, it was found that granulomas and *M. leprae* were present in the skin and lymph node biopsies of all patients except two, in whom, although granulomas persisted, *M. leprae* were not found in skin biopsy specimens. No growth was obtained in the foot pads of mice inoculated with organisms isolated from skin and lymph node biopsies of all 11 patients, indicating a near complete bacterial kill. That would account for the extremely low relapse rates reported until now in LL patients who had undergne 2 years of MDT.—Authors' Abstract

Vora, N. S., Vora, V. N., Mukhopadhyay, A. K., Roy, K., Patel, N. N. and Ghosh, A. A case of histoid leprosy responding to ofloxacin along with standard MDT. Indian J. Lepr. **67** (1995) 183–186.

The 50-year-old man in India with histoid leprosy had not been treated previously. The lesions disappeared completely after his treatment with ofloxacin for 28 days, followed by standard multidrug therapy to ensure eradication of the infection.—Authors' Abstract

Wadee, A. A., Kuschke, R. H., Dooms, T. G. and Anderson, R. The pro-oxidative riminophenazine B669 neutralized the inhibitory effects of *Mycobacterium tuberculosis* on phagocyte antimicrobial activity. Int. J. Immunopharmacol. **17** (1995) 849–856.

The effects of clofazimine, a riminophenazine antimicrobial agent, and its analog B669 on phagocyte functions have been investigated. Clofazimine, at concentrations attainable *in vivo*, and B669, in particular, increased the intracellular killing ability of phagocytes following appropriate cell stimulation. Similarly, nitro blue tetrazolium reduction, hydrogen peroxide production, lysosyme release and hexose monophosphate shunt activity were all increased by treating phagocytes with the riminophenazines. It has previously been shown that a 25 kDa glycolipoprotein derived from *Mycobacterium tuberculosis* inhibits phagocyte functions associated with phagocyte antimicrobial activity. The present study confirms these observations. A further aspect of the study examined the ability of riminophenazines to reverse the inhibition of phagocyte functions by the 25 kDa mycobacterial fraction. While both riminophenazines were capable of partially but significantly reversing the inhibition due to the mycobacterial fraction, the restorative capacity of B669 was greater than that of clofazimine.—Authors' Abstract

Clinical Sciences

Arunthathi, S., Samuel, J., Ebenezer, G. J. and Jacob, M. Localized borderline lepromatous leprosy. Indian J. Lepr. **67** (1995) 177–181.

The authors provide a case report for a 17-year-old man in India who presented with two skin lesions localized to the right upper arm and showed histopathological features of borderline lepromatous leprosy, but without any nerve enlargement.—Trop. Dis. Bull.

Chen, J. [Initial symptoms in 318 cases of leprosy.] China Lepr. J. **11** (1995) 195–196. (in Chinese)

Initial symptoms were investigated in 318 leprosy patients with the age of 5 to 84 years and a mean age of 34 years, including 245 men and 73 women, and 215 PB and 103 MB. In terms of their frequency, they in turn were numbness (36.5%), erythema (26.1%), numb macula (14.5%), neuralgia (21.1%) and paresthesia (10.4%).—Author's English Abstract

Ribeiro de Carvalho, M. de L., Arujo, M. G., Guedes, A. C. M. and Patrus. O. A. [An evaluation of borderline tuberculoid leprosy during multidrug therapy.] An.

Bras. Dermatol. **70** (1995) 201–204. (in Portuguese)

The authors describe the clinical evolution of 71 cases of borderline tuberculoid (BT) leprosy undergoing multidrug therapy (MDT) in Minas Gerais, Brazil (studied from August 1989 to August 1993). The clinical picture was rated as "mild" (up to 5 skin lesions) or "severe" (more than 5 skin lesions) and, as to the histological picture, BT patients were grouped as having "high" or "low" immunity. The evolution of these two groups during MDT showed no statistically significant differences. The lepromin reaction (Mitsuda) was shown to be determinant in the evolution of BT patients.—Authors' English Abstract

Ribeiro de Carvalho, M. de L., Arujo, M. G., Guedes, A. C. M. and Patrus, O. A. [Type 1 reaction in borderline tuberculoid leprosy under multidrug therapy: time of onset and nerves affected.] An. Bras. Dermatol. **70** (1995) 205–208. (in Portuguese)

The authors describe the onset of type 1 reaction and the affected nerves in 71 patients from Minas Gerais, Brazil (examined in August 1989-August 1993) undergoing multidrug therapy (MDT) for borderline tuberculoid (BT) leprosy; 89.3% of the BT patients with type 1 reaction developed their symptoms before the sixth dose of MDT. The ulnar nerve was the most frequently affected, occurring in 37% of cases of type 1 reaction.—Authors' English Abstract

Silva, C. M. P., Silva, A. C. M., Marques, S. C., Saldanha, A. C. R., Nascimento, J. D. L., Brancho, M. R. F. C., Silva, R. R. and Costa, J. M. L. [Association of chromoblastomycosis and leprosy: two case reports.] Rev. Soc. Bras. Med. Trop. **27** (1994) 241–244. (in Portuguese)

Thirty cases of chromoblastomycosis were diagnosed at the Hospital dos Servidores do Estado do Maranhão, Brazil, between November 1988 and March 1993. The authors report on two (6.6%) cases that presented an association with leprosy. The first patient developed both diseases together, showing palpable bilateral cubital nerves, perforating ulcer of the right foot, infiltration and lesions in verrucoid plaques in the left leg, with a positive biopsy for dimorphic leprosy. The second case was in a patient with history of lepromatous leprosy for 30 years without treatment, with vegetant lesions with a warty aspect on the right elbow for 12 months, and with histopathological and positive culture for chromoblastomycosis. The possible factors for development of this disease in these patients are discussed.—Authors' English Abstract

Soni, N. K. Leprosy of the eustachian tube (nasopharyngoscopic study). Lepr. Rev. **66** (1995) 314–317.

The technique of nasopharynogoscopy affords an accurate assessment of the lesions at the orifices of the eustachian tube. It was performed in 30 patients suffering from lepromatous leprosy in order to determine the type, nature and site of the lesion. Involvement of the eustachian tube in leprosy may begin with a localized area of erythema progressing to granuloma formation or ulceration. Leprous lesion at the eustachian tube orifices was related with subsequent changes in the tympanogram pattern. Nasopharyngoscopy is also found to be of therapeutic value in removing the crust, discharge and granulations at the eustachian tube orifices.—Author's Summary

Torre Alba, J., Mendoza, I., Ocanto, T., Barroeta, S., Mejia de Alejos, M. A. and Bonfante-Garrido, R. Concomitant cutaneous leishmaniasis and leprosy in Venezuela. Trans. R. Soc. Trop. Med. Hyg. **89** (1995) 69.

A case is reported of a 42-year-old male patient, resident in a peripheral region of Barquisimeto, Venezuela, near the Turbio River, presenting concomitant clinical characteristics of both cutaneous leishmaniasis and lepromatous leprosy. The leishmaniasis lesion healed in 7 weeks after meglumine antimonate (25 mg/kg for 15 consecutive days, repeated after an interval of 1 week). He received long-term treatment with clofazimine and rifampin for leprosy. The negative Montenegro for leishmaniasis in this patient was believed to be due to the short period during which he had been infected with leishmaniasis (<3 months) and to the promptness with which specific treatment was started. It is emphasized that since

leprosy and cutaneous leishmaniasis have a number of similar clinical characteristics, skin smears should be taken from several lesions when the coexistence of the two infections is suspected.—Authors' Abstract

Wang, J.-Q., *et al.* [Silent neuritis in persons cured of leprosy with DDS monotherapy.] China Lepr. J. **11** (1995) 184–185. (in Chinese)

Since 1992 to 1994 in Dongtai City, Jiangsu, out of 1028 persons cured of leprosy with monotherapy 9 cases of silent neuritis (0.88%) were found, which involved 12 nerves, including 1 facial, 1 median, 2 ulnar, 4 tibial and 4 common peroneal nerves. They all occurred 13 to 24 years, with a mean time of 17.7 years, after their leprosy had been cured.—Authors' English Abstract

Yan, L., *et al.* [A matched survey of ophthalmia in leprosy.] China Lepr. J. **11** (1995) 177–181. (in Chinese)

Ophthalmopathy was surveyed by matching among 1045 cases of leprosy in Taixing City. The rate of ocular diseases was 63.92%, and most of the lesions were in the anterior part of eyeball and periocular area. Dysopsia rate was 27.18%, and blindness made up 12.07%. The causes of dysopsia were cataract in 41.2%, keratopathy in 33.45% and iridophathy in 17.25%. In the matched controls ophthalmopathy mostly was senile, the rate of ocular diseases was 9.95% and dysopsia rate was 1.53%. The authors suggest that for prevention of ophthalmopathy in leprosy the oculists must take part.—Authors' English Abstract

Immuno-Pathology

Baird, M. A., Hart, D. N. J., Abernethy, N. and Watson, J. D. Dendritic cell presentation of PPD and 19 kDa protein of *Mycobacterium tuberculosis* and emergent T helper cell phenotype. Immunol. Cell Biol. **73** (1995) 537–543.

Protection against infection with *Mycobacterium tuberculosis* is preferentially associated with the development of the T-helper 1 subset, IFN-gamma production and a cell-mediated response, rather than with T-helper 2 cells, 4 (IL-4) and antibody production. The type of APC interacting with T cells responsive to mycobacterial peptides may influence which of these responses predominates. This investigation focuses on the role of dendritic cells (DC) because they are the most potent APC in both primary and recall immune responses. Our results show that splenic DC-enriched suspensions prepared from C57BL/6 mice and pulsed with either purified protein derivative (PPD) or the immunodominant 19-kDa protein from *M. tuberculosis*, can activate antigen-primed T cells *in vitro*; whereas spleen cell suspensions depleted of DC cannot. DC pulsed with PPD or 19-kDa antigen are able to prime naive T cells *in vivo*. Supernatants collected from cultures containing T cells from mice injected with PPD-pulsed DC and then challenged *in vitro* with PPD-pulsed DC were found to contain more IL-2 and IFN-gamma than those from control mice which received either DC or PPD alone. No such antigen-specific IFN-gamma response occurred if DC pulsed with 19-kDa were used in place of PPD-pulsed DC. IL-4 was not detected in any of the culture supernatants. We conclude that DC can induce production of cytokines associated with a protective immune response when presenting peptides derived from heterogeneous mycobacterial antigens but not when exposed to the single 19-kDa immunodominant protein.—Authors' Abstract

Bakos, L. and Lucas, S. B. Immunohistochemical study of cutaneous neuritis in positive lepromin reactions. Lepr. Rev. **66** (1995) 277–286.

Sixty skin biopsies taken from positive tuberculoid and borderline tuberculoid late lepromin reactions were studied using histological techniques. The distribution of mycobacterial antigen and nerves was demonstrated using immunochemical methods.

A total of 557 nerve bundles was observed in 51 biopsies; 9 were devoid of nerves in the sections examined; 475 nerve bundles showed some relationship to the

inflammatory infiltrate (85%); perineuritis being seen in 144 (30%) and endoneuritis in 5 (0.9%).

Mycobacterial antigens inside the granuloma were detected in 59 of the 60 biopsies (98%). Only one specimen, showing a strong tuberculoid reaction, failed to show these antigens. On the contrary, mycobacterial antigen was absent in almost all nerves. Small deposits were detected in the perineurium of one nerve with perineuritis, and inside a Schwann cell of another, the latter belonging to a previously multibacillary patient.

The neurotropic tendency of the granuloma does not seem to be stimulated by the presence of mycobacterial antigens inside the nerves, as normally these antigens do not penetrate them. The hypothesis of some antigenic fraction of the neural tissue which crossreacts with *Mycobacterium leprae* antigens, thus eliciting a perineural or near-perineural inflammatory reaction is put forward, but needs further investigation.—Authors' Summary

Bernhagen, J., Bacher, M., Calandra, T., Metz, C. N., Doty, S. B., Donnelly, T. and Bucala, R. An essential role for macrophage migration inhibitory factor in the tuberculin delayed-type hypersensitivity reaction. J. Exp. Med. **183** (1996) 277–282.

Thirty years ago, investigations into the molecular basis of the delayed-type hypersensitivity (DTH) reaction provided evidence for the first lymphokine activity: a lymphocyte-derived mediator called macrophage migration inhibitory factor (MIF), which inhibited the random migration of peritoneal macrophages. Despite the long-standing association of MIF with the DTH reaction and the cloning of a human protein with macrophage migration inhibitory activity, the precise role of MIF in this classic cell-mediated immune response has remained undefined. This situation has been further complicated by the fact that two other cytokines, interferon gamma and IL-4, similarly inhibit macrophage migration and by the identification of mitogenic contaminants in some preparations of cloned human MIF. Using recently developed molecular probes for mouse MIF, we have examined the role of this protein in a classical model of DTH, the tuberculin reaction in mice. Both MIF messenger RNA and protein were expressed prominently in DTH lesions, as assessed by reverse transcription polymerase chain reaction, *in situ* hybridization, and immunostaining with anti-MIF antibody. The predominant cellular origin of MIF appeared to be the monocyte/macrophage, a cell type identified recently to be a major source of MIF release *in vivo*. The administration of neutralizing anti-MIF antibodies to mice inhibited significantly the development of DTH, thus affirming the central role of MIF in this classic immunological response.—Authors' Abstract

Burroughs, M. H., Tsenova Berkova, L., Sokol, K., Ossig, J., Tuomanen, E. and Kaplan, G. Effect of thalidomide on the inflammatory response in cerebrospinal fluid in experimental bacterial meningitis. Microb. Pathogen. **19** (1995) 245–255.

In experimental bacterial meningitis in rabbits, the inflammatory process is largely mediated by cytokines such as IL-1 and TNF-alpha. Since thalidomide has been shown to inhibit TNF-alpha production, experiments were carried out to determine whether the drug can modulate the inflammatory response to either lysates of *H. influenzae* (gram negative) or heat-killed *S. pneumoniae* (gram positive) in rabbits. The introduction of a lysate of *H. influenzae* into the CSF of rabbits causes a very acute inflammatory response, as indicated by a rapid increase in TNF-alpha levels in the CSF and a concomitantly rapid leukocytosis. In contrast, the introduction of heat killed *S. pneumoniae,* induces a more indolent inflammatory response which also wanes more slowly. Thalidomide treatment reduces TNF-alpha production in both experimental systems, but has a greater effect on the more indolent gram-positive inflammatory response in which peak TNF-alpha levels in the CSF are reduced by >50%. Also, a sustained inhibition of leukocytosis is observed in the inflammatory response to heat-killed gram-positive bacteria. In meningeal inflammation induced by the gram-negative lysate, treatment with thalidomide results in only a 29% inhibition of TNF-alpha release into the CSF. In contrast to the drug

effect on TNF-alpha, thalidomide treatment does not significantly affect IL-1 levels in these models of rabbit bacterial meningitis.—Authors' Abstract

Joko, S., Numaga, J., Fujino, Y., Masuda, K., Hirata, R. and Maeda, H. [HLA-DR2 alleles and uveitis in leprosy.] Jpn. J. Lepr. **64** (1995) 112–118. (in Japanese)

In order to investigate the role of immunogenetic factors in the pathogenesis of uveitis in leprosy, HLA antigens were analyzed in 65 Japanese leprosy patients: 32 with uveitis and 33 without uveitis. Controls consisted of 138 healthy subjects. A lymphocyte cytotoxicity test was used for typing HLA-A,-B,-C,-DR and-DQ antigens. HLA-DR2 genotyping was performed by the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and PCR-restriction fragment length polymorphism (RFLP) methods. The frequency of HLA-DR2 antigen was significantly increased in the patients with uveitis as compared with the control subjects ($pc < 0.0005$); whereas HLA-DR53 antigen was significantly decreased ($pc < 0.05$). At the genomic level the frequency of HLA-DRB1*1501 was significantly increased in the patients with uveitis and without uveitis as compared with that in the controls. The association with DRB1*1501 was even stronger in patients with uveitis (odds ratio = 7.1, $pc < 0.000005$) than in the patients without uveitis (odds ratio = 4.1, $pc < 0.005$). The authors' results suggest that HLA-DRB1*1501 contributes to the susceptibility to uveitis in the Japanese with leprosy.—Authors' English Abstract

Kumaravel, M. and Megha Singh. Aggregation and deformability of erythrocytes in leprosy. Indian J. Exp. Biol. **33** (1995) 408–415.

The hemorheological parameters, erythrocyte aggregation and deformability are determined in leprotic patients and are compared with values for healthy subjects. The aggregation is determined by sequential analysis of the He-Ne laser transmission data through erythrocyte suspension at hemotocrit 5%. The erythrocyte deformability is determined by measurement of passage time (reciprocal of deformability) of erythrocyte suspension in PBS at hematocrit 6% through cellulose membrane. The observations show that in leprosy the aggregation of erythrocyte is marginally reduced and the deformability is significantly increased. These parameters in combination with low hemoglobin and hematocrit levels in these patients lower the blood viscosity to maintain the transport of material across the capillary wall.—Authors' Abstract

Mesret, Y., Reed, A. H. and Howe, R. C. Proliferative responses of T cells from the skin and nerve lesions of leprosy patients. Clin. Immunol. Immunopathol. 77 (1995) 243–252.

In the current study we compared the mitogenic responses of T cells from skin and nerve biopsies of leprosy patients with those of peripheral blood mononuclear cells (PBMC). Lymphocytes from these sources were cultured at ≤ 100 cells/well in the presence of PHA, irradiated autologous feeder cells, and IL-2, and proliferation was assessed after 6 to 12 days. Whereas PBMC were capable of vigorous responses, the growth of cells from skin and nerve was markedly reduced. The diminished response was independent of the clinical status of leprosy patients and was also observed in skin-infiltrating lymphocytes from patients suffering from other disorders. Analysis of proliferative responses at 1 cell/well suggested both a reduction in precursor frequency and a decrease in mean burst size. Analysis of lymphokine production suggested that cultured cells from skin lesions had reduced IL-2 and IL-4 production relative to PBMC generated under similar conditions. Equal numbers of CD3+ cells were present in each source, but lesion cells were enriched in CD45RA(−) "memory" T cells, as well as CD3(+)CD28(+) T cells. However, these alterations in subpopulation distribution could not account for the substantial differences in proliferative potential. We conclude that significant differences exist in the activation potential of cells from different tissue sources.—Authors' Abstract

Misra, N., Selvakumar, M., Singh, S., Bharadwaj, M., Ramesh, V., Misra, R. S. and Nath, I. Monocyte derived IL-10 and

PGE_2 are associated with the absence of Th1 cells and *in vitro* T cell suppression in lepromatous leprosy. Immunol. Lett. **48** (1995) 123–128.

Our previous studies had shown that the clinicopathological spectrum in leprosy was associated with discrete T cell subsets in the circulation, with tuberculoid patients having antigen-induced Th1; whereas lepromatous leprosy patients with antigen-specific T-cell anergy possesed Th2 cells. The present study shows that infected monocytes from lepromatous but not tuberculoid leprosy patients released soluble factors (MoFs) containing IL-10 and PGE_2 which inhibited *M. leprae* induced *in vitro* lymphoproliferation of previously sensitized healthy or tuberculoid leprosy subjects. A strong negative correlation was observed between adherant-cell-derived IL-10 and IL-2 at the level of both the product and cytokine mRNA. Moreover, anti-IL-10 antibodies and indomethacin partially reversed the suppressor effects of MoFs. Taken together these studies indicate that infected monocytes contribute to the development of T-cell anergy by releasing factors that affect regulatory cytokines and T-cell subset differentiation in lepromatous leprosy.—Authors' Abstract

Mustafa, A. S. Identification of mycobacterial peptide epitopes recognized by CD4+ T cells in association with multiple major histocompatibility complex class II molecules. Nutrition **11** Suppl. 5 (1995) 657–660.

The mycobacterial 18-, 28-, and 65-kDa proteins are recognized by T cells in association with multiple class II HLA-DR molecules of the major histocompatibility complex (MHC). To identify the epitopes recognized by T cells in association with multiple HLA-DR molecules, we established CD4+ T-cell lines and clones and tested them with overlapping synthetic peptides corresponding to the entire amino-acid sequence of the 18- and 65-kDa antigens and to the carboxy terminus of the 28-kDa antigen. The T-cell lines established against the 18-kDa antigen recognized three different epitopes, one of which was recognized in the presence of antigen-presenting cells from several allogeneic donors. The 65-kDa antigen-reactive T-cell lines responded to nine different peptides, two of which were promiscuous with respect to MHC restriction. The T-cell clones responding to the 28-kDa antigen proliferated in response to a single peptide from the carboxy terminus. This peptide was recognized by T-cell clones in association with HLA-DRw53, which is coexpressed in individuals expressing HLA-DR4, HLA-DR7, and HLA-DR9. The T cells activated in response to the mycobacterial antigen from vaccinated donors belonged to the protective Thl subset; hence, the peptides recognized in association with multiple HLA-DR molecules should be useful in subunit vaccines against mycobacterial diseases.—Author's Abstract

Mustafa, A. S. Isolation and characterization of the genes of pathogenic mycobacteria that express antigens for T cell reactivity. Nutrition **11** Suppl. 5 (1995) 653–656.

Tuberculosis and leprosy are caused by *Mycobacterium tuberculosis* and *M. leprae,* respectively. Identification and characterization of the genes expressing proteins that stimulate Th1-type cells is required to understand the mechanisms involved in protection from mycobacterial diseases. We isolated several recombinant genes from recombinant DNA libraries that expressed antigens recognized by well-characterized monoclonal antibodies. We first used these isolated recombinants to show that many of these genes expressed antigens with T-cell reactivity. Most of the genes in the lambda gtll system were truncated at the amino terminus. Full-length genes were isolated from libraries in other systems and sequenced. Four of the full-length genes were homologous to heat-shock proteins of eukaryotes and prokaryotes. We also screened the recombinant DNA libraries directly with T-cell probes for antigens that may not be recognized by antibodies. We isolated one gene expressing an epitope recognized by T cells reactive with *M. tuberculosis, M. leprae,* and *M. bovis* BCG but not with other mycobacteria. All recombinant proteins were presented to T cells in association with multiple HLA-DR molecules. The responding T cells were the Th1 type with long-lasting memory. The protein products of

these genes, either by themselves or expressed in suitable vaccines, may be used to protect against tuberculosis and leprosy.—Author's Abstract

Mustafa, A. S. *Mycobacterium bovis* BCG-induced Th1-type CD4+ suppressor T cells act by suppressing IL-2 production and IL-2 receptor expression. Nutrition **11** Suppl. 5 (1995) 692–694.

Induction of suppressor cells has been hypothesized to explain the variability in the efficacy of *Mycobacterium bovis* BCG vaccination against mycobacterial diseases. In this study, we induced suppressor T cells by *in vitro* stimulation of peripheral blood mononuclear cells obtained from BCG-vaccinated healthy subjects. These suppressor T cells were CD4+ and did not affect interleukin-1 (IL-1) production by adherent cells in response to BCG. However, they suppressed IL-2 production and IL-2 receptor expression by the responding cells. Exogenous addition of IL-2 could partially restore the responsiveness of the indicator cells. To further characterize the cells responsible for suppression, T-cell clones were established by limiting dilution. All the established T-cell clones expressed CD4 marker, proliferated in response to BCG, were cytotoxic for antigen-presenting cells, and suppressed the antigen-induced proliferation of the indicator cells. Both suppression and cytotoxicity were not mediated by soluble factors but required cell-to-cell contact and were HLA-class II restricted. These results suggest that preferential killing of antigen-presenting cells by CD4+ T cells may be responsible for *in vitro* observed suppression in our system.—Author's Abstract

Mustafa, A. S. Recognition of mycobacterial hsp65 in association with HLA-DR4 is not sufficient for autoreactivity. Nutrition **11** Suppl. 5 (1995) 661–664.

The mycobacterial heat-shock protein 65, class II major histocompatibility complex molecule HLA-DR4, and T cells have been implicated in autoimmunity. However, there has been no direct demonstration of the recognition of the heat-shock protein 65 by T cells in association with HLA-DR4. In this study, we established T-cell lines and a large number of T-cell clones from healthy subjects vaccinated with killed mycobacteria. Among these subjects, three were HLA-DR4 positive, and the T-cell lines and clones from these subjects responded to the mycobacterial heat-shock protein 65. HLA-restriction studies were done with well-characterized anti-HLA class I, anti-HLA-DR, and anti-HLA-DQ antibodies. Only anti-HLA-DR antibodies inhibited the antigen-induced response of the T-cell lines and clones to heat-shock protein 65. When HLA-DR-typed allogeneic cells were used as antigen-presenting cells, the T-cell clones from only one of the individuals were found to be HLA-DR4 restricted. To identify the epitopes recognized by these T-cell clones, synthetic peptides were synthesized covering the entire sequence of mycobacterial heat-shock protein 65. When tested with the heat-shock protein 65-reactive T-cell clones, peptides from five different regions of heat-shock protein 65 stimulated the T-cell clones in association with HLA-DR4. However, it is difficult to predict the role of HLA-DR4-restricted mycobacterial heat-shock protein 65 peptides in autoimmunity from our studies, because the T-cell clones were established from a healthy donor, and the peptides belonged to the regions that do not share sequence homology between the mycobacterial and human heat-shock protein 65 or other proteins.—Author's Abstract

Mustafa, A. S., Daschugh, T. and Abal, A. T. Polymerase chain reaction targeting of single- and multiple-copy genes of mycobacteria in the diagnosis of tuberculosis. Nutrition **11** Suppl. 5 (1995) 665–669.

Tuberculosis is a worldwide health problem of major concern. Direct detection of *Mycobacterium tuberculosis* in clinical specimens is the best approach to identify the causative agent. Identification of *M. tuberculosis* by culture is the gold standard, but the results are delayed for days to weeks. Microscopic examination of smears is quite fast, but a sample must contain a large number of *M. tuberculosis* ($>7.5 \times 10^3$ organisms/ml) for smear positivity. To diagnose tuberculosis specifically within 1 day of receiving clinical specimens, we have established multiplex polymerase chain reaction (MPCR) assays by targeting DNA frag-

ments in the genes present in single or multiple copies in the *M. tuberculosis* genome. The MPCR results are available within a few hours, and the detection limit for different targets ranges between 2 and 200 organisms. The targets selected in the MPCRs could differentiate between *M. tuberculosis* complex and other mycobacteria from culture-grown specimens. The MPCRs were compared with microscopic examination of smears and culture in the diagnosis of tuberculosis. Coded sputum samples from suspected tuberculosis patients were tested. The codes were broken at the end of the study and the results were compared. All of the samples negative for smear and/or culture were also negative by multiple-copy gene MPCRs (specificity = 100%); whereas the single-copy gene MPCR showed 98% specificity. With respect to sensitivity, compared with culture, the single-copy gene MPCR showed a sensitivity of 92%; whereas the three- and two-band multiple-copy gene MPCRs exhibited sensitivities of 87% and 93%, respectively. These results suggest that the MPCRs could be helpful in early and specific diagnosis of pulmonary tuberculosis.—Authors' Abstract

Mustafa, A. S. and Oftung, F. Cytokine production and cytotoxicity mediated by CD4+ T cells from healthy subjects vaccinated with *Mycobacterium bovis* BCG and from pulmonary tuberculosis patients. Nutrition **11** Suppl. 5 (1995) 698–701.

In tuberculosis, T cells are responsible for protection but also the pathology caused by inflammatory responses. Most T cells activated in response to *Mycobacterium tuberculosis* express the CD4 phenotype and are divided into Th1 and Th2 subsets, depending on the types of cytokines produced. Th1 cells protect against most intracellular infections, including tuberculosis. To study the Th1 and Th2 profiles against *M. tuberculosis* antigens, we established CD4+ T-cell clones from the peripheral blood mononuclear cells of healthy subjects vaccinated with *M. bovis* BCG and of pulmonary tuberculosis patients. When tested for cytokine production in response to mycobacterial antigens and defined epitopes (i.e., whole killed *M. tuberculosis*, a 65-kDa heat-shock protein, and synthetic peptides), the T-cell clones produced cytokines typical of Th1 cells: interleukin 2, interferon-gamma, and granulocyte-macrophage colony-stimulating factor. The same T cells also had cytotoxic activity against antigen-pulsed macrophages. We propose that activation of macrophages by interferon-gamma and killing of the pathogen-laden macrophages by cytotoxic T cells may contribute to protection. However, the same mechanisms may also activate the release of soluble mediators responsible for inflammatory responses seen in tuberculosis granulomas.—Authors' Abstract

Neubert, R., Hinz, N., Thiel, R. and Neubert, D. Down-regulation of adhesion receptors on cells of primate embryos as a probable mechanism of the teratogenic action of thalidomide. Life Sci. **58** (1995) 295–316.

In spite of ongoing speculation, there has been no evidence that adhesion receptors are expressed on the cells of mammalian embryos. In this report, we provide the first proof that a variety of such receptors (beta 1-, beta 2-, and beta 3-integrins and selectin) are indeed expressed on cells of essentially all primordia of marmoset embryos at early organogenesis (developmental stages 11 to 13, or even earlier).

Treatment with low doses (20 or as little as 1 mg/kg body weight) of a highly teratogenic derivative (EM12) of thalidomide, the most notorious human teratogen, triggers a dramatic and statistically highly significant down-regulation of several surface adhesion receptors (e.g., CD11a/CD18, CD49d/CD29, CD61, etc.) on early limb bud cells and on cells of some other primordia during early organogenesis of embryos of a primate (marmoset, *Callithrix jacchus*). Some of these receptors almost disappear, or they are expressed at a lower epitope density in the exposed embryos. These down-regulations of surface adhesion receptors may be expected to alter cell-cell- and cell-extracellular matrix interactions, and they are suggested to be a long-sought primary mechanism of the teratogenic action of thalidomide-type substances.—Authors' Abstract

Nogueira, A. C., Neubert, R., Felies, A., Jacobmuller, U., Frankus, E. and Neubert,

D. Thalidomide derivatives and the immune system. 6. Effects of two derivatives with no obvious teratogenic potency on the pattern of integrins and other surface receptors on blood cells of marmosets. Life Sci. **58** (1995) 337–348.

The two thalidomide (Thd) derivatives beta-EM12 and phthalimidophthalimide (Phtpht), which exhibit no obvious teratogenicity, were tested for their ability to induce changes in the pattern of lymphocyte subpopulations, and especially changes in integrin receptors, in marmosets (*Callithrix jacchus*). Previously, Thd and its highly teratogenic derivative alpha-EM12 had been found to alter the expression of adhesion molecules, such as CD2 (LFA-2) or CD11a/CD18 (LFA-1). None of these typical effects on adhesion receptors were observed following administration of the relatively high daily doses of 50 mg/kg body wt beta-EM12 and Phtpht.

Nevertheless, there were some minor effects, such as alterations in the receptor density on peripheral blood mononuclear cells, which were often contrary to the effects induced by Thd. Mainly affected were: CD8 cells, B cells bearing the CD54 receptor and CD4 cells bearing the CD56 (NCAM) surface marker. We observed an increase in the receptor density of CD11c (p150, 95) on monocytes with Phtpht but not with beta-EM12.

The inability of the two substances with no obvious teratogenic potential to typically modify beta 2-integrin receptors on white blood cells at comparatively high doses is consistent with our hypothesis that the teratogenicity of Thd may also be linked to alterations in the expression of adhesion molecules.—Authors' Abstract

Ramaprasad, P., Cree, I. A., Oluwole, M. and Samson, P. D. Development of a mucosal challenge test for leprosy using leprosin A. J. Immunol. Methods **188** (1995) 239–246.

There is little information about the mucosal immune response in leprosy. We have developed a nasal provocation test with leprosin A which will be used to investigate mucosal immunity to *Mycobacterium leprae*. Initial studies were performed with increasing doses of leprosin A (1.0 pg/ml-10 μg/ml) to determine the optimal safe dose of leprosin A. Anti-*M. leprae* IgA antibody and normal IgA concentrations were measured in the saliva of leprosy contacts and controls before and after instillation of leprosin A. Nasal leprosin A was well tolerated up to a concentration of 10 μg/ml without side effects. None of the six subjects who had not been exposed to leprosy had salivary IgA against whole *M. leprae*; whereas IgA was detected from 64 hr to 140 hr following instillation of leprosin A in all of the leprosy hospital workers and in 15 out of 18 healthy household contacts tested. There was no correlation between serum and salivary anti-*M. leprae* IgA levels before and after testing. Salivary IgA anti-lipoarabinomannan responses were seen in 12 out of 20 household contacts. Normal salivary IgA concentrations varied from 8 to 240 mg/l. The leprosin A nasal provocation test appears to be a safe method for the investigation of the role of mucosal immunity in the pathogenesis of leprosy.—Authors' Abstract

Roche, P. W., Winter, N., Triccas, J. A., Feng, C. G. and Britton, W. J. Expression of *Mycobacterium tuberculosis* MPT64 in recombinant *Myco. smegmatis:* purification, immunogenicity and application to skin tests for tuberculosis. Clin. Exp. Immunol. **103** (1996) 226–232.

Proteins secreted across the cell wall of mycobacteria are important antigens recognized early in the host response to mycobacterial infection. MPT64 is a 23-kD secreted protein restricted to members of the *Mycobacterium tuberculosis* complex which elicits T-cell responses and cutaneous DTH reactions in *M. tuberculosis*-infected animals. Patients with tuberculosis and their tuberculin-positive contacts respond to the protein, but recipients of bacille Calmette-Guerin (BCG) vaccine strains lacking the mpt64 gene do not. In the present study, we describe the development of a unique recombinant mycobacterial vector which secretes the encoded *M. tuberculosis* protein MPT64 at high levels into the culture filtrate, from which the protein is isolated by a single-step affinity chromatographic step. The purified protein was recognized by both polyclonal and monoclonal anti-MPT64 antibodies. The T-cell reactivity of the pro-

tein was confirmed by its ability to stimulate human anti-rMPB64 T-cell lines. The *M. smegmatis* recombinant MPT64 protein was superior to the *Escherichia coli* rMPB64 protein, which has identical amino acid sequence, in eliciting cutaneous DTH reactions in guinea pigs sensitized with *M. tuberculosis*. Animals sensitized with BCG strains lacking the mpb64 gene failed to respond to MPT64. Similarly, interferon-gamma responses in tuberculosis patients and their contacts were higher to the *M. smegmatis* form of the protein. The potential of this form of the *M. tuberculosis* MPT64 protein as a skin-test reagent for tuberculosis is discussed.—Authors' Abstract

Saad, M. H. F., Gormus, B. J., Cho, S.-N., Bernheimer, H. and Schwerer, B. Detection if IgA anti-PGL-I specific antigen to *Mycobacterium leprae* in mangabey monkeys inoculated with *M. leprae*. Lepr. Rev. **66** (1995) 296–306.

Using sera from four pairs of mangabey monkeys inoculated with titrated doses of *Mycobacterium leprae*, we demonstrated that IgA antibodies against *M. leprae*-specific PGL-I antigen were present in 75% of inoculated monkeys' sera. High IgA antibody was detected in 50% (3/6) of infected animals and all 3 developed lepromatous leprosy (LL). Antibody titers correlated with PGL-I antigen in serum. The highest IgA peak appeared late and corresponded to the beginning of treatment, and in 2 of them appeared shortly after or corresponded with neurological damage. Low IgA response was found in the other 3 monkeys (50%-3/6), 2 of which developed indeterminate leprosy (I) and the other one LL. Low IgA levels appeared late after IgG and IgM, and shortly after neurologic signs. Both I monkeys were negative for PGL-I in serum. The remaining 2 monkeys (25%-2/8) did not show an IgA response; one of them developed LL but the disease regressed to I. IgM seemed to correspond to the appearance of PGL-I in serum. The other animal did not develop clinical symptoms of leprosy, and PGL-I in serum was negative.

Although there was no clear relation between the development of anti-PGL-I IgA and experimental leprosy, the finding of a high IgA response in some animals suggests that further studies are needed to evaluate the role of antigen-specific IgA in the disease process.—Authors' Summary

Scheel Toellner, D., Richter, E., Toellner, K. M., Reiling, N., Wacker, H. H., Flad, H. D. and Gerdes, J. CD26 expression in leprosy and other granulomatous diseases correlates with the production of interferon-gamma. Lab. Invest. **73** (1995) 685–690.

Background: Leprosy represents a spectrum of clinical manifestations that reflect the immune response to antigens of *Mycobacterium leprae*. The tuberculoid form of leprosy, which is characterized by an organized development of granulomas, has recently been correlated with a Th1-like immune response. The lepromatous form of leprosy, with a characteristic lack of cellular immunity, has been correlated with a Th2-like immune response to mycobacterial antigens. Dipeptidylpeptidase IV (CD26) is an ectopeptidase that is expressed in various tissues; in the hemopoietic system, it is predominantly expressed by T cells.

Experimental design: We stained frozen sections of skin biopsies obtained from patients with different forms of leprosy, sarcoidosis, and Piringer's lymphadenitis. Sections were stained for interferon-gamma (IFN-γ) and CD26 with the alkaline phosphatase anti-alkaline phosphatase technique and in two-color stainings by immunofluorescence.

Results: We found strong signals for IFN-γ and for CD26 in all investigated cases of tuberculoid leprosy. In contrast, in all biopsies taken from patients with lepromatous leprosy, we found no or very weak signals for these antigens. By immunofluorescence double-labeling, we could show that IFN-γ and CD26 were expressed by the identical cell population. We confirmed this correlation of CD26 expression and IFN-γ production in other granulomatous inflammatory reactions, such as sarcoidosis and Piringer's lymphadenitis.

Conclusions: From our results, we conclude that a high expression of CD26 may be suggestive of Th1-like immune reactions.—Authors' Summary

Shannon, E. J. and Sandoval, F. Thalidomide increases the synthesis of IL-2 in

cultures of human mononuclear cells stimulated with concanavalin-A, staphylococcal entertoxin A, and purified protein derivative. Immunopharmacology **31** (1995) 109–116.

Thalidomide significantly increases the quantity of extracellular IL-2 in cultures of human mononuclear cells stimulated with mitogens or antigen. Cells from 7 donors exposed for 2 hr to 4.0 μg/ml of thalidomide and stimulated for 16-18 hr with 20 μg/ml of concanavalin-A (ConA) averaged producing 187 ± 49% more IL-2 than cells stimulated with ConA alone. In similar experimental procedures and comparisons the pg/ml of IL-2 secreted by thalidomide-treated cells from 5 donors stimulated with 50 ng/ml of Staphylococcal enterotoxin A (SEA) increased by 159 ± 32%, and the pg/ml of IL-2 secreted by thalidomide-treated cells from 2 donors stimulated with 5.0 μg/ml of purified protein derivative of *Mycobacterium tuberculosis* increased by 120 ± 4%. Thalidomide also significantly increases the quantity of intracellular IL-2 in cells stimulated with mitogens. Cells exposed to thalidomide and stimulated with ConA had an increase in intracellular IL-2 of 130% after 8 hr and 157% after 12 hr in culture; cells stimulated with SEA had an increase in intracellular IL-2 of 120% after 8 hr and 182% after 12 hr in culture. Thalidomide did not alter the percent of lymphocytes expressing the α-chain of IL-2 receptor, nor did it significantly increase incorporation of ^{3}H-thymidine by cells.—Authors' Abstract

Shetty, V. P., Mistry, N. F., Birdi, T. J. and Antia, N. H. Effect of T-cell depletion of bacterial multiplication and pattern of nerve damage in *M. leprae*-infected mice. Indian J. Lepr. **67** (1995) 363–374.

Various mechanisms for nerve damage in tuberculoid leprosy have been proposed. A common feature among them is the crucial role played by T cells. Therefore, the present study was designed to determine the role of T cells in the induction of nerve damage in leprosy using two different protocols for obtaining graded levels of T-cell depletion: (i) cyclosporine A, for depletion of T-helper cells and (ii) anti-Thy 1.2, for total depletion of T cells. The findings indicate that the early changes seen in the unmyelinated fibers may not involve T cells. However, the later stages of nerve damage associated with demyelination are dependent on T-cell responses.—Authors' Abstract

Silva, C. L., Pietro, R. L. R., Januario, A., Bonata, V. L. D., Lima, V. M. P., Dasilva, M. F. and Lowrie, D. B. Protection against tuberculosis by bone marrow cells expressing mycobacterial hsp65. Immunology **86** (1995) 519–524.

Although mice acquire only a slight degree of protection against tuberculosis by immunization with *Mycobacterium leprae* hsp65 in incomplete Freund's adjuvant, protection is substantial following immunization by injection with J774 macrophage-like tumour cells that express the protein from the mycobacterial gene via a retroviral vector. We here took the same vector, used it to transfect the gene into normal murine bone marrow cells *in vitro*, and then used the transfected cells to reconstitute hematopoiesis in lethally irradiated mice. Bone marrow-cell clonal expansion and production of the protein *in vivo* resulted in specific delayed-type hypersensitivity and protection against challenge with *M. tuberculosis* in about half of recipients. Counts of live bacteria in liver at 3 weeks were fivefold lower in delayed-type hypersensitivity (DTH)-positive than in DTH-negative mice. Other mice acquired neither DTH nor protection despite the presence of the protein in peripheral blood.—Authors' Abstract

Struyk, L., Hawes, G. E., Haanen, J. B. A. G., de Vries, R. R. P. and Vandenelsen, P. J. Clonal dominance and selection for similar complementarity determining region 3 motifs among T lymphocytes responding to the HLA-DR3-associated *Mycobacterium leprae* heat shock protein 65-kd peptide 3-13. Hum. Immunol. **44** (1995) 220–227.

In order to establish whether specific MHC class II-peptide complexes are capable of selecting TCR V regions, we investigated in detail the TCR beta chain used in the recognition of HLA-DR3 restricted hsp65 peptide 3-13 in a tuberculoid leprosy patient. Using RT-PCR, a clear dominance of the TCRBV5 gene family was observed in a hsp65 peptide 3-13-specific T-cell line;

however, not in fresh, unstimulated PBMCs, PHA-stimulated PBMCs, or a T-cell line specific for tetanus toxoid.

DNA sequence analysis of the TCR V regions, comprising TCRBV5 genes, derived from the hsp65 peptide 3-13-specific T-cell line revealed the exclusive usage of the TCRBV5S1 gene segment and a predominance of one V-D-J gene rearrangement, which is indicative of clonal expansion of these T lymphocytes. Additional highly similar V-D-J gene rearrangements were detected at a low level in this hsp65 peptide 3-13-specific T-cell line. These conserved junctional regions (CDR3 regions) could not be detected within the TCRBV5 gene family of fresh PBMCs, PHA-stimulated PBMCs, hsp65, and tetanus-toxoid-specific T-cell lines from this patient.

The observations in this tuberculoid leprosy patient reveal that an HLA class-II-restricted T-cell response results in selection of TCRBV regions which are highly similar in amino acid composition to the CDR3 region within the expanding TCRBV regions.—Authors' Abstract

Microbiology

Desikan, K. V. and Sreevatsa. Extended studies on the viability of *Mycobacterium leprae* outside the human body. Lepr. Rev. **66** (1995) 287–295.

Very little is known in leprosy regarding the transmission of the infection from the source to the susceptible host. One of the important factors which governs the transmission of the disease is the viability of *Mycobacterium leprae* outside the human body. In this study *M. leprae* obtained from untreated patients have been subjected to several adverse conditions. Their viability was verified by their multiplication in the foot pads of normal mice. After drying in the shade the organisms were viable up to 5 mos. On wet soil, they remained alive for 46 days. Kept in saline at room temperature, the organisms lived for 60 days. Surprisingly on exposure to direct sunlight for 3 hr a day the bacteria survived for 7 days. On refrigeration at 4°C, the bacteria could be preserved for 60 days. On the other hand, keeping at −70°C, the bacteria could be maintained in a living condition for only 28 days. On exposure to antiseptics like Savlon® and alcohol, the bacteria were rapidly killed. These results indicate the survival outside the human body of *M. leprae* under different environmental conditions in India where the disease is endemic. Transmission of infection by indirect contact and occurrence of new cases in the absences of any known source, are consistent with *M. leprae* being viable outside the human body for varying periods of time. The findings could also be pointers to understand the epidemiology of leprosy.—Authors' Summary

Gonzalez y Merchand, J. A., Estrada-Garcia, I., Colston, M. J. and Cox, R. A. A novel method for the isolation of mycobacterial DNA. FEMS Microbiol. Lett. **135** (1996) 71–77.

DNA was isolated from mycobacteria by a simplified procedure. Cells were suspended in 6 M guanidinium chloride, the suspension was cooled to −70°C, then incubated at 65°C for 10 min, cooled in ice, deproteinized by chloroform and DNA was recovered from the supernatant. The procedure was used to obtain DNA from several mycobacteria (1×10^9 or more cells) including *Mycobacterium neoaurum, M. fortuitum, M. phlei* and *M. smegmatis.* Each of the species was shown to have two ribosomal RNA operons per genome, and preliminary evidence was obtained which suppests that one of these operons is homologous with one of the operons of *M. smegmatis.*—Authors' Abstract

Madhusudan, K. and Nagaraja, V. *Mycobacterium smegmatis* DNA gyrase: cloning and overexpression in *Escherichia coli.* Microbiology (U.K.) **141** Part 12 (1995) 3029–3037.

The cloning and characterization of DNA gyrase genes from *Mycobacterium smegmatis* is described. The DNA sequence of 5119 bp encoding both gyrB and gyrA genes

was determined. The gene gyrB precedes gyrA with a short intergenic region of 29 nucleotides. The proteins encoded, GyrB and GyrA, exhibit 45%-80% identity to gyrase polypeptides from other bacteria. The genes were further engineered for overexpression in *Escherichia coli*. Both genes were individually cloned into a phage T7 expression system and overexpressed. The expressed GyrB and GyrA proteins had molecular masses of 75 and 95 kDa, respectively, in agreement with that calculated from the ORFs. The extracts from the overexpressing clones were fractionated to enrich the subunits and assayed for enzyme activity. While the individual extracts showed no detectable activity, the combined extract exhibited a strong DNA supercoiling activity. This activity was ATP-dependent and novobiocin-sensitive. The identity of the genes was also confirmed by complementation analysis.—Authors' Abstract

Mande, S. C., Mehra, V., Bloom, B. R. and Hol, W. G. J. Structure of the heat shock protein chaperonin-10 of *Mycobacterium leprae*. Science **271** (1996) 203–207.

Members of the chaperonin-10 (cpn10) protein family, also called heat shock protein 10 and in *Escherichia coli* GroES, play an important role in ensuring the proper folding of many proteins. The crystal structure of the *Mycobacterium leprae* cpn10 (MI-cpn10) oligomer has been elucidated at a resolution of 3.5 angstroms. The architecture of the MI-cpn10 heptamer resembles a dome with an oculus in its roof. The inner surface of the dome is hydrophilic and highly charged. A flexible region, known to interact with cpn60, extends from the lower rim of the dome. With the structure of a cpn10 heptamer now revealed and the structure of the *E. coli* GroEL previously known, models of cpn10:cpn60 and GroEL:GroES complexes are proposed.—Authors' Abstract

Mehrotra, J., Bisht, D., Tiwari, V. D. and Sinha, S. Serological distinction of integral plasma membrane proteins as a class of mycobacterial antigens and their relevance for human T cell activation. Clin. Exp. Immunol. **102** (1995) 626–634.

This study pertains to classification and antigenic analysis of mycobacterial plasma membrane proteins in relation to human T-cell proliferative responses, using a "fast grower" *Mycobacterium fortuitium* as model. Membrane vesicles, prepared by sonication and differential centrifugation, were subjected to biphasic Triton X-114 extraction for isolation of integral (detergent phase) and peripheral (aqueous phase) proteins. Neither protein pool showed any appreciable overlap serologically. SDS-PAGE showed five prominent bands in peripheral and three in the integral protein pool; whereas immunoblotting with rabbit antisera identified only two major antigens (60 and 67 kDa) in the former and five (24, 34, 42, 51 and 54 kDa) in the latter. ELISA with a panel of antimycobacterial MoAbs revealed that 9 out of 12 previously known antigens were present in the peripheral protein pool. Only two of them (33 and 40 kDa) were additionally detected among integral proteins. The membrane-associated immunosuppressive moiety lipoarabinomannan was semiquantitatively located in aqueous phase. In bulk T-cell proliferation assays, 7 out of 10 subjects belonging to a "responder" background (BT-BB leprosy patients and healthy contacts) showed high responses for *M. fortuitium* antigens. Proliferative response with integral proteins was comparable to that with whole membrane, but it was significantly higher ($p < 0.0005$) than the response with peripheral proteins. The distinction and relevance of integral membrane proteins as a class of mycobacterial antigens make them worthy of consideration in a subunit vaccine design.—Authors' Abstract

Menendez, M. C., Domenech, P., Prieto, J. and Garcia, M. J. Cloning and expression of the *Mycobacterium fortuitum* superoxide dismutase gene. FEMS Microbiol. Lett. **134** (1995) 273–278.

In this paper we report the cloning, sequencing and expression of the superoxide dismutase (sod) gene from *Mycobacterium fortuitum*. A single gene was found to code for superoxide dismutase activity with its identity being confirmed by expression in *M. aurum*. The amino acid sequence was found to be similar to that of superoxide

dismutases of several other origins. A region downstream of the sod gene also showed similarities to the corresponding sequences of the two main mycobacterial pathogens: *M. leprae* and *M. tuberculosis.* Analysis of enzymatic activity showed this enzyme in *M. fortuitum* required manganese as cofactor.—Authors' Abstract

Scherman, M., Weston, A., Duncan, K., Whittington, A., Upton, R., Deng, L., Comber, R., Friedrich, J. D. and McNeil, M. Biosynthetic origin of mycobacterial cell wall arabinosyl residues. J. Bacteriol. **177** (1995) 7125–7130.

Designing new drugs that inhibit the biosynthesis of the D-arabinan moiety of the mycobacterial cell-wall arabinogalactan is one important basic approach for treatment of mycobacterial diseases. However, the biosynthetic origin of the D-arabinosyl monosaccharide residues themselves is not known. To obtain information on this issue, mycobacteria growing in culture were fed glucose labeled with ^{14}C or ^{3}H in specific positions. The resulting radiolabeled cell walls were isolated and hydrolyzed, the arabinose and galactose were separated by high-pressure liquid chromatography, and the radioactivity in each sugar was determined. [U-^{14}C]glucose, [6-^{3}H]glucose, [6-^{14}C]glucose, and [1-^{14}C]glucose were all converted to cell-wall arabinosyl residues with equal retention of radioactivity. The positions of the labeled atoms in the arabinose made from [1-^{14}C]glucose and [6-^{3}H]glucose were shown to be C-1 and H-5, respectively. These results demonstrated that the arabinose carbon skeleton is formed via the nonoxidative pentose shunt and not via hexose decarboxylation or via triose condensations. Since the pentose shunt product, ribulose-5-phosphate, is converted to arabinose-5-phosphate as the first step in 3-keto-D-manno-octulosonic acid biosynthesis by gram-negative bacteria, such a conversion was then searched for in mycobacteria. However, cell-free enzymatic analysis using both phosphorous nuclear magnetic resonance spectrometry and colorimetric methods failed to detect the conversion. Thus, the conversion of the pentose shunt intermediates to the D-*arabino* stereochemistry is not via the expected isomerase but rather must occur via novel metabolic transformations.—Authors' Abstract

Takiff, H. E., Cimino, M., Musso, M. C., Weisbrod, T., Martinez, R., Delgado, M. B., Salazar, L., Bloom, B. R. and Jacobs, W. R. Efflux pump of the proton antiporter family confers low-level fluoroguinolone resistance in *Mycobacterium smegmatis.* Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 362–366.

Due to the resurgence of tuberculosis and the emergence of multidrug-resistant strains, fluoroquinolones (FQ) are being used in selected tuberculosis patients, but FQ-resistant strains of *Mycobacterium tuberculosis* have rapidly begun to appear. The mechanisms involved in FQ resistance need to be elucidated if the effectiveness of this class of antibiotics is to be improved and prolonged. By using the rapid-growing *M. smegmatis* as a model genetic system, a gene was selected that confers low-level FQ resistance when present on a multicopy plasmid. This gene, lfrA, encodes a putative membrane efflux pump of the major facilitator family, which appears to recognize the hydrophilic FQ, ethidium bromide, acridine, and some quaternary ammonium compounds. It is homologous to qacA from *Staphylococcus aureus,* tcmA of *Streptomyces glaucescens,* and actII and mmr both from *Streptomyces coellcoler.* Increased expression of lfrA augments the appearance of subsequent mutations to higher-level FQ resistance.—Authors' Abstract

Thole, J. E. R., Wieles, B., Clark Curtiss, J. E., Ottenhoff, T. H. M., Rinke, T. F. and de Wit, F. R. Immunological and function characterization of *Mycobacterium leprae* protein antigens: an overview. Mol. Microbiol. **18** (1995) 790–800.

A major focus of leprosy research in the last 10 years has been the identification and characterization of antigens of *Mycobacterium leprae* that interact with antibodies and T cells of the host's immune response. Through the combined efforts of many different laboratories, a substantial number of protein antigens have been identified and characterized. In this microreview we present an updated list of *M. leprae* protein an-

tigens and, with emphasis on recent developments, summarize what is known regarding their functional and immunological features.—Authors' Abstract

Wieles, B., Nagai, S., Wiker, H. G., Harboe, M. and Ottenhoff, T. H. M. Identification and functional characterization of thioredoxin of *Mycobacterium tuberculosis.* Infect. Immun. **63** (1995) 4946–4948.

We have previously described a *Mycobacterium tuberculosis* protein designated MPT46 that was present in culture filtrates. Here we report that the MPT46 protein is thioredoxin of *M. tuberculosis.* MPT46 is recognized by antibodies to thioredoxin (Trx) of *Escherichia coli,* and antibodies to MPT46 recognize *M. leprae* Trx. Moreover, MPT46 was shown to have enzymatic activity identical to that of Trx of other species, such as its ability to reduce insulin. These findings identify MPT46 as a functionally active Trx.—Authors' Abstract

Wieles, B., Spierings, E., Vannoort, J., Naafs, B., Offringa, R. and Ottenhoff, T. Molecular characterization and T-cell-stimulatory capacity of *Mycobacterium leprae* antigen T5. Infect. Immun. **63** (1995) 4682–4685.

A *Mycobacterium leprae* lambda gt11 clone designated T5 has previously been selected with sera from tuberculoid leprosy patients. Sequence analysis of this clone revealed the presence of two overlapping open reading frames (ORFs) present on the two cDNA strands. The first ORF codes for the serologically recognized antigen, which was fused with the lacZ gene in the lambda gt11 clone. The second ORF, present on the complementary strand, displays strong sequence homology with the aspartyl-tRNA synthetase genes of *Escherichia coli* and *Thermus thermophilus.* Here we show that the purified T5-derived product, overexpressed in *E. coli,* is recognized by T cells of the majority of the leprosy patients tested, including lepromatous leprosy patients who do not respond to whole *M. leprae* bacilli.—Authors' Abstract

Wiese, M. and Seydel, U. Monitoring of drug effects on cultivable mycobacteria and *Mycobacterium leprae* via the determination of their adenylate energy charges (AEC). J. Microbiol. Methods **24** (1995) 65–80.

The adenylate energy charges (AEC) of *mycobacterium smegmatis, M. tuberculosis* and *M. leprae* were determined for an untreated and drug-treated bacterial sample. AEC values of the cultivable mycobacteria ranged between 0.66–0.71 in exponentially growing cultures and decreased below 0.1 under the influence of various drugs and drug combinations depending on concentration. AEC values of *M. leprae* samples isolated from different armadillo tissues and from untreated multibacillary leprosy patients were significantly lower (0.32–0.52). For *M. leprae* isolated from patients after 3 months of effective multidrug therapy bacterial impairment could be monitored with this technique. For differently treated cultures of *M. smegmatis* and *M. tuberculosis,* a linear correlation between AEC and viability as derived from the mass spectrometric analysis of the intrabacterial sodium to potassium ratio of single bacterial organisms was established within a certain AEC range. The range in which AEC is linearly correlated with viability, however, was found to be dependent on the mode of drug action and the bacterial species.—Authors' Abstract

Wu, L. C. C. and Shahied, S. I. Mycobacterial DNA gyrase: enzyme purification and characterization of supercoiling activity. Arch. Biochem. Biophys. **324** (1995) 123–129.

Putative structural genes encoding *Mycobacterium bovis* BCG gyrase A and gyrase B subunits were expressed in *Escherichia coil* under the control of a regulated promoter. Upon induction, high levels of proteins of M(r) 92,000 and 75,000 were generated. Purification and reconstitution of these proteins yielded an enzyme with bacterial DNA gyrase activity. DNA supercoiling activity of the mycobacterial enzyme required ATP, Mg2+, and spermidine. Like other bacterial DNA gyrases, the supercoiling activity of the mycobacterial enzyme was inhibited by low concentration of the classical gyrase B subunit inhibitors novo-

biocin and coumermycin. Older gyrase A subunit inhibitors, nalidixic and oxolinic acid, had no effect on the supercoiling activity at 400 to 800 μg/ml. However, *in vitro* assays to show the inhibition of supercoiling activity and stimulation of cleavable complex formation demonstrated that ciprofloxacin is a potent inhibitor of mycobacterial DNA gyrase. The availability of highly purified mycobacterial DNA gyrase could aid in future investigations of quinolone derivatives targeting *Mycobacterium* specifically.—Authors' Abstract

Epidemiology and Prevention

de Andrade, V. L. G., Sabroza, P. C. and de Arujo, A. J. G. [Factors associated with household and family in leprosy transmission in Rio de Janeiro, Brazil.] Cardenos Saude Publica **10** Suppl. 2 (1994) 281–292. (in Portuguese)

A cross-sectional study was carried out to characterize the contribution of several household characteristics to the transmission of leprosy. Randomly selected households with diagnosed cases of illness in the municipality of São Gonçalo, in the state of Rio de Janeiro, Brazil, were compared with two healthy groups. Using an aerial map with the description of the census tract, 2412 cases were marked. Three groups were established, with the household as the analytical unit: Group I, households with cases; Group II, neighboring households; and Group III, households located in tracts with no reported cases of illness, i.e., outside disease foci. Group I was compared with the neighboring households using a multiple logistic regression model by conditional methods. Unconditional methods were used to compare Groups I and III. Group I as compared with Group II showed an association with age and educational level for households and heads of families. Comparison of characteristics of the heads of families and households with cases of leprosy with those located outside the focus showed that the differential factors were age, type of dwelling, and availability of running water. This finding is probably due to more recent settlement in a peripheral region where water resources are not yet available. Households are the basic ecological unit, and age and educational level are determinant factors for leprosy morbidity in this area.—Authors' English Abstract

Liu, S., *et al.* [New cases of leprosy in the last 10 years in Xuzhou City, Jiangsu Province (China).] China Lepr. J. **11** (1995) 199–200. (in Chinese)

Xuzhou City, Jiangsu Province, has a population of 8, 300,000 and had accumulated 1617 cases of leprosy until the end of 1980. In the period of 1981 to 1990, 177 new cases of leprosy were detected, including 128 men and 49 women with proportion of 1:2. 32 in MB to PB, a mean disease duration of 3.1 years and a mean age of 39.8 years. Disability (over Grade II) rate was 36.16%.—Authors' English Abstract

Pandhi, R. K., Khanna, N. and Sekhri, R. Leprosy settlement colonies of Delhi. Indian J. Lepr. **67** (1995) 467–471.

Population influx into urban areas like Delhi has encouraged mushrooming of numerous slums where about 30% population of the city is living. A survey was conducted in four resettlement colonies of Delhi. Of the 6876 persons examined, 43 (6.25 per 1000) subjects were found to have clinical and histologic evidence of leprosy. Fifteen (35%) patients of neuritic leprosy, 8 (19%) with tuberculoid leprosy, 12 (38%) of borderline tuberculoid, 3 (4%) each with borderline and borderline lepromatous and 1 (2%) each of lepromatous and indeterminate leprosy were diagnosed. The study revealed that 21% of the patients were less than 20 years of age.—Authors' Abstract

Stanimirovic, A., Skerlev, M., Gacina, P., Beck, T., Stipic, T., Basta-Juzbasic, A. Leprosy in Croatia in the twentieth century. Lepr. Rev. **66** (1995) 318–323.

Even today, leprosy is a relatively frequently occurring disease, especially in tropical regions of the world. From the 11th to 13th century, leprosy pandemics affected Europe, including Croatia. Probably as a consequence of such history, one can still find endemic foci of leprosy in present-day Croatia.

The aim of this study was to analyze all cases of leprosy registered in Croatia during the 20th century; therefore, we studied thoroughly existing medical documentation and published reports on sporadic leprosy cases, and went on to collect the relevant data through on-site investigation in those parts of Croatia known as putative endemic foci of leprosy. In this way, we collected data concerning the number of leprosy cases, the probable sources of infection, and traced the possible paths of spread of the disease.

During the 20th century, 17 cases of leprosy were registered in Croatia. However, due to the loss of medical documentation concerning the cases from Metković, the total number was obviously slightly greater. Concerning the 17 analyzed cases, 4 patients were most probably infected during their visits (as sailors or immigrant workers) to the Middle East, South America or Africa; 3 patients developed leprosy after prolonged close contact with previously infected family members, while the exact source of infection remains unsettled for the remaining 10. However, 2 of these patients originated from the area of Cazin in Bosnia and Herzegovina, which is known to be an endemic focus of leprosy. Furthermore, the remaining 8 came from the small area of the village of Blizna in the Croatian municipality of Trogir, and therefore it seems reasonable to conclude that Blizna represents the endemic focus of leprosy in Croatia. The last case of leprosy in Blizna was registered back in 1956. Nevertheless, it is clear that sporadic cases of leprosy can reappear in Croatia, originating either from this endemic focus of Blizna, or as an infected person returning to Croatia from abroad. So, we can conclude that, even today, Croatian medical doctors (and especially dermatovenereologists) should still be acquainted with the clinical diagnosis of leprosy and basic principles of its treatment.—Authors' Summary

Sterne, J. A. C., Ponnighaus, J. M., Fine, P. E. M. and Malema, S. S. Geographic determinants of leprosy in Karonga District, Northern Malawi. Int. J. Epidemiol. **24** (1995) 1211–1222.

Background. Geographical differences in leprosy risk are not understood, but may provide clues about the natural history of the disease. We report an analysis of the geographical distribution of leprosy in Karonga District, a rural area of northern Malawi, between 1979 and 1989.

Methods. Cohort study of the incidence of leprosy based on two total population surveys. Area of residence was determined using aerial photographs, which allowed identification of households, as well as location of roads, rivers and the lake shore.

Results. Incidence rates were between two and three times higher in the north compared to the south of the district, and lowest in the semi-urban district capital. The most obvious environmental difference between these regions is the north's higher rainfall and more fertile soil. There was no overall association between leprosy incidence and population density, although highest rates were observed in the least densely populated areas. Looking at the entire district, incidence rates increased with increasing distance from a main road, but declined with increasing distance from a river or from the shore of Lake Malawi. The negative association with proximity to rivers may reflect the larger number of rivers in the north of the district. Apparent differences in incidence rates between groups speaking different languages reflected confounding by area of residence.

Conclusions. There is a marked variation, not explained by socioeconomic or cultural factors, in the incidence of leprosy within Karonga District. Our results are consistent with a theme in the literature associating the environment, particularly proximity to water, with leprosy.—Authors' Abstract

Wang, R. [Effects of leprosy control in Sichuan.] China Lepr. J. **11** (1995) 193–194. (in Chinese)

Sichuan Province has begun to control leprosy since the 1950s. After over 40 years, the number of the patients decreased from

40,000 and more to 2861, the prevalence from 0.67 to 0.026 per 1000, and the incidence among children reduced to 0.001/100,000. In 16 counties the detection rate was below 0.2/100,000, so there are 152 counties to reach or approach the target of basic eradication of leprosy.—Author's English Abstract

Zhou Y., *et al.* [Impact of MDT on endemicity of leprosy in Yunnan.] China Lepr. J. **11** (1995) 187–188. (in Chinese)

After wide implementation of short-term MDT recommended by WHO for 6 years, in Yunnan Province yearly mean detecting rate decreased from 2.14/100,000 to 1.68/100,000, incidence from 1.62/100,000 to 1.03/100,000 and proportions of children and disabled ones with grade II and more among newly detected patients are still so high as 3.42% and 22.9%, respectively. The authors hold that the case-finding should still be the main work for leprosy control there hereafter.—Authors' English Abstract

Rehabilitation

Ethiraj, T., Antony, P., Krishnamurthy, P. and Reddy, N. B. B. A study on the effect of patient and community education in prevention of disability programme. Indian J. Lepr. **67** (1995) 435–446.

The effect of self-care learning by leprosy patients in prevention of disabilities was studied by adapting two strategies in two subcenters of a project in South India, one through patient education by trained field staff and the other through community education involving trained animators and health committees. One of the subcenters was taken as control where neither of the strategies was employed. In terms of results, though both the strategies were found to be effective in containing occurrence of new deformities among high-risk patients and healing of trophic ulcers in hands and feet, strategy I, i.e., self-care education of patients by concerned field personnel without prejudice to their routine work is recommended because of ease in diffusion of strategy.—Authors' Abstract

Li, B.-Y. [On marriage situation of the relatives of leprosy patients.] China Lepr. J. **11** (1995) 197–198. (in Chinese)

The marriage situation among 744 sons-daughters and 1177 brothers-sisters of 550 leprosy patients were surveyed in Sichuan Province. There was no difference of marriage rates between the sons-daughters and controls, but the rate was significantly lower in the brothers-sisters. The divorce rate in the relatives of the patients was very significantly higher than in the controls, indicating that discrimination against leprosy still is very serious there.—Author's English Abstract

Mahajan, P. M., Jogaikar, D. G. and Mehta, J. M. Study of deformities in children with leprosy: an urban experience. Indian J. Lepr. **67** (1995) 405–425.

A study was conducted to assess the deformities in children with leprosy. Eyes, hands and feet were examined for leprosy-related deformities. The influence of age, sex, duration of disease, type of disease, occurrence of leprosy reactions and antileprosy treatment on the occurrence of deformities was studied. In our urban leprosy project the percentage of children (0–14 years) suffering from leprosy is 7% of the total number of leprosy patients. The response to multidrug therapy, health education and physiotherapy was good.—Authors' Abstract

Mahajan, P. M., Kulkarni, V. N., Jadhav, V. H. and Mehta, J. H. Wax therapy for dry feet in leprosy. Indian J. Lepr. **67** (1995) 383–388.

A comparative study of the effect of wax therapy and foot soaks on dry plantar skin was conducted in patients with leprosy. Thirty patients with varying grades of fissures and callosities were given wax therapy for feet, and 20 similar patients were given foot soaks. Patients given wax therapy felt subjectively much better than those who had

soaking. Healing of cracks and fissures and softening of callosities was observed more frequently in patients with wax therapy. These differences are statistically significant. As an institutional method, wax therapy has definite advantages for treating patients with fissures and callosities; whereas soaking of the feet is easy and readily available in patients' homes to restore the dry skin to normal.—Authors' Abstract

Raju, M. S. and Reddy, J. V. S. Community attitude to divorce in leprosy. Indian J. Lepr. **67** (1995) 389–403.

Divorcing a leprosy-afflicted spouse is one of the manifestations of social stigma attached to leprosy. It mostly depends on the community's decision resulting from the physical and social threat perceived. In order to find out who were prone to divorce their leprosy-afflicted spouses, 1199 community members drawn from two states, Orissa and Andhra Pradesh, were asked what their advice would be if a spouse of a leprosy patient approached them for advice. The responses were crosstabulated against their demographic characteristics. While only a small proportion of respondents advised divorce in Andhra Pradesh, they were mainly females, above SSC educated, those who did cultivation, laborers, and were from poor economic group. On the other hand, in Orissa a high proportion of the respondents suggested divorce.—Authors' Abstract

Ramarorazana, S., Rene, J. P., Schwartzl, E., Randrianomenjanahary, J. Razafindramboa, H. and di Schino, M. [One-year followup of 466 nerve decompression procedures in 123 lepers [sic] during multidrug therapy in Madagascar.] Med. Trop. **55** (1995) 146–150. (in French)

At the leprosy center of Ambatoabo on the east coast of Madagascar, 466 nerve decompressions were performed on 123 subjects and results were evaluated by the same examiner 15 months later. All subjects were undergoing multidrug therapy at the time of the procedure. The decision to perform surgery was based on recent onset of sensory and motor neurological signs and on progression or persistence of symptoms despite administration of prednisolone. Decompression led to pain relief in 100% of cases and regression of sensory disturbances in 97%. Sensory recovery in the plantar ulcers of the foot was obtained in 80% of cases and motor performance improved in 61%. These favorable results confirm the value of surgical decompression to prevent sequels of leprosy and the feasibility of this procedure in remote areas.—Authors' English Abstract

Soares, D. Tibialis posterior transfer for the correction of foot drop in leprosy. J. Bone Joint Surg. Br. **78B** (1996) 61–62.

A comparison was made of the results produced by the circumtibial and interosseous routes of transfer of tibialis posterior for the correction of foot drop due to leprosy neuritis. The findings in 69 feet, of which 63 also had elongation of tendo Achillis, showed that the interosseous route gave a much lower incidence of recurrent inversion deformity of the foot. The results, in terms of improvement in gait and prevention of trophic changes, were satisfactory.—Author's Abstract

Other Mycobacterial Diseases and Related Entities

Avanis Saghanjani, E., Jones, K., Holtzman, A., Aronson, T., Glover, N., Boian, M., Froman, S. and Brunk, C. F. Molecular technique for rapid identification of mycobacteria. J. Clin. Microbiol. **34** (1996) 98–102.

Identification of mycobacteria through conventional microbiological methods is cumbersome and time-consuming. Recently we have developed a novel bacterial identification method to accurately and rapidly identify different mycobacteria directly from water and clinical isolates. The method utilizes the PCR to amplify a portion of the small subunit rRNA from mycobacteria. The 5′ PCR primer has a fluorescent label to allow detection of the amplified product.

The PCR product is digested with restriction endonucleases, and an automated DNA sequencer is employed to determine the size of the labeled restriction fragments. Since the PCR product is labeled only at the 5′ end, the analysis identifies only the restriction fragment proximal to the 5′ end. Each mycobacterial species has a unique 5′ restriction fragment length for each specific endonuclease. However, frequently the 5′ restriction fragments from different species have similar or identical lengths for a given endonuclease. A set of judiciously chosen restriction enzymes produces a unique set of fragments for each species, providing us with an identification signature. Using this method, we produced a library of 5′ restriction fragment sizes corresponding to different clinically important mycobacteria. We have characterized mycobacterial isolates which had been previously identified by biochemical tests and/or nucleic acid probes. An analysis of these data demonstrates that this protocol is effective in identifying 13 different mycobacterial species accurately. This protocol has the potential of rapidly (less than 36 hr) identifying mycobacterial species directly from clinical specimens. In addition, this protocol is accurate, sensitive, and capable of identifying multiple organisms in a single sample.—Authors' Abstract

Balasubramanian, V., Pavelka, M. S., Bardarov, S. S., Martin, J., Weisbrod, T. R., McAdam, R. A., Bloom, B. R. and Jacobs, W. R. Allelic exchange in *Mycobacterium tuberculosis* with long linear recombination substrates. J. Bacteriol. **178** (1996) 273–279.

Genetic studies of *Mycobacterium tuberculosis* have been greatly hampered by the inability to introduce specific chromosomal mutations. Whereas the ability to perform allelic exchanges has provided a useful method of gene disruption in other organisms, in the clinically important species of mycobacteria, such as *M. tuberculosis* and *M. bovis,* similar approaches have thus far been unsuccessful. In this communication, we report the development of a shuttle mutagenesis strategy that involves the use of long linear recombination substrates to reproducibly obtain recombinants by allelic exchange in *M. tuberculosis.* Long linear recombination substrates, approximately 40 to 50 kb in length, were generated by constructing libraries in the excisable cosmid vector pYUB328. The cosmid vector could be readily excised from the recombinant cosmids by digestion with PacI, a restriction endonuclease for which there exist few, if any, sites in mycobacterial genomes. A cosmid containing the mycobacterial leuD gene was isolated, and a selectable marker conferring resistance to kanamycin was inserted into the leuD gene in the recombinant cosmid by interplasmid recombination in *Escherichia coli.* A long linear recombination substrate containing the insertionally mutated leuD gene was generated by PacI digestion. Electroporation of this recombination substrate containing the insertionally mutated leuD allele resulted in the generation of leucine auxotrophic mutants by homologous recombination in 6% of the kanamycin-resistant transformants for both the Erdman and H37Rv strains of *M. tuberculosis.* The ability to perform allelic exchanges provides an important approach for investigating the biology of this pathogen as well as developing new live-cell *M. tuberculosis*-based vaccines.—Authors' Abstract

Barnes, P. F., El-Hajj, H., Preston Martin, S., Cave, M. D., Jones, B. E., Otaya, M., Pogoda, J. and Eisenach, K. D. Transmission of tuberculosis among the urban homeless. JAMA **275** (1996) 305–307.

Objective—To determine the relative frequencies of primary and reactivation tuberculosis in the urban homeless.

Design—Prospective evaluation of homeless tuberculosis patients.

Setting—Central Los Angeles, California, U.S.A.

Patients—Thirty-four homeless patients with culture-proven tuberculosis.

Interventions—IS61 10-based restriction fragment length polymorphism (RFLP) analysis was performed on *Mycobacterium tuberculosis* isolates. If results were inconclusive, pTBN12-based RFLP analysis was performed.

Main Outcome Measure—Clustering of *M. tuberculosis* isolates. A cluster consisted of two or more isolates with indistinguishable RFLP patterns.

Results—Twenty-four of 34 homeless patients had clustered isolates in six clusters.

Conclusions—The minimum percentage of cases due to primary tuberculosis in the homeless was estimated to be 53%, compared with the traditional estimate of 10% in the general population. The results suggest that primary tuberculosis caused the majority of tuberculosis cases in this population of the urban homeless in central Los Angeles.—Authors' Abstract

Basu, A., Mistry, N. F. and Antia, N. H. Views on the sustenance of resistant *Mycobacterium tuberculosis* in the environment. Med. Hypotheses **45** (1995) 421–426.

Microbial resistance to conventional as well as newly introduced drugs is a hallmark feature of several infectious diseases, notably tuberculosis. It is hypothesized that the greater the selective pressure exerted by increasingly potent drugs, the more rapidly is an organism able to adapt to a drug-containing environment. The roles of drug-containing environments, and the immunological status of the host and bacterial molecular mechanisms of development of drug resistance to *Mycobacterium tuberculosis* have been examined and examples cited for implementation of modified drug regimens in tuberculosis-control programs. The views expressed, albeit restricted to *M. tuberculosis,* encourage consideration of drug regimens on a disease evolution basis as well as understanding of the natural rules that govern development and sustenance of drug resistance in the microbial world.—Authors' Abstract

Degitz, K. Detection of mycobacterial DNA in the skin: etiologic insights and diagnostic perspectives. Arch. Dermatol. **132** (1996) 71–75.

Background: Tuberculosis may be as old as mankind and continues to be a serious medical problem today. Cutaneous tuberculosis shows considerable morphological variability, and it is included in the differential diagnosis of many other skin disorders. It is especially difficult to distinguish skin tuberculosis from other granulomatous processes of the skin. Therefore, reliable laboratory tests are needed to confirm or rule out the diagnosis. However, the diagnostic identification of *Mycobacterium tuberculosis* and related organisms has remained difficult using conventional laboratory tests (i.e., microscopy and culture).

Observations: The diagnostic usefulness of molecular techniques, especially the polymerase chain reaction (PCR), in skin tuberculosis is reviewed, and the technical issues of PCR in general are discussed, with special regard to the analysis of mycobacterial DNA in skin specimens. The PCR has been successfully applied to detect DNA from *M. tuberculosis* in lupus vulgaris and several other forms of skin tuberculosis. It has also been used to identify mycobacterial DNA in certain forms of tuberculids, thereby supporting the long- and often-debated tuberculous origin of these skin disorders. Investigations of the presence of mycobacterial DNA in cutaneous sarcoidosis have not lent support to a general role for mycobacteria in sarcoidosis.

Conclusions: Polymerase chain reaction-based detection of *M. tuberculosis* DNA in skin samples may extend and improve the diagnostic panel for cutaneous tuberculosis, if the technique is prudently and properly used. Furthermore, PCR provides exciting opportunities to gain further insight into the pathogenesis of cutaneous tuberculosis and other granulomatous skin diseases.—Author's Abstract

D'Souza, S., Levin, M., Faith, A., Yssel, H., Bennett, B., Lake, R. A., Brown, I. N. and Lamb, J. R. Defective antigen processing association with familial disseminated mycobacteriosis. Clin. Exper. Immunol. **103** (1996) 35–39.

To gain insights into a possible immune defect predisposing to disseminated mycobacteria infection, we studied three of six surviving children with disseminated *Mycobacterium avium* complex infection, who had no recognized form of immunodeficiency. We used mycobacteria isolated from the patients and diphtheria, tetanus and pertussis vaccine (DTP) to study antigen-specific T-lymphocyte responses. We observed that interferon-gamma (IFN-γ) production by T cells in response to antigens (both mycobacteria and DTP) in these patients with disseminated infection was greatly impaired. This defect did not seem to be the result of T-cell unresponsiveness, as phytohemagglutinin (PHA) stimulation

was able to induce high levels of IFN-γ comparable to those seen in control patients with localized infection. Further experiments showed that peripheral blood mononuclear cells (PBMC) from patients with disseminated infection were able to present influenza hemagglutinin (HA) peptides to specific T-cell clones. However, this ability was lost when the whole HA protein was used as source of antigen. Taken together, these observations support the notion that the primary immune defect in these patients with disseminated mycobacterial infection rests in the antigen-processing functions of their antigen-presenting cells (APC). These findings may provide clues to the wider problem of susceptibility to mycobacteria and other intracellular pathogens and have implications in designing therapy for these patients.—Authors' Abstract

Harboe, M., Oettinger, T., Wiker, H. G., Rosenkrands, I. and Andersen, P. Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. Infect. Immun. **64** (1996) 16–22.

ESAT-6 is a secreted protein present in the short-term culture filtrate of *Mycobacterium tuberculosis* after growth on a synthetic Sauton medium. ESAT-6 has recently been demonstrated to induce strong T-cell responses in a mouse model of memory immunity after infection with *M. tuberculosis.* In Western blotting (immunoblotting), the monoclonal antibody HYB76-8, reacting with ESAT-6, gave a 6-kDa band in culture filtrates from *M. tuberculosis* and virulent *M. bovis.* A distinct band in the 24-kDa region was observed in filtrates from four of eight substrains of *M. bovis* BCG that produced high levels of MPB64, while no band occurred in the 6-kDa region with any of these BCG substrains. southern blotting and PCR experiments with genomic mycobacterial DNA showed the presence of the esat-6 gene in reference strains and clinical isolates of *M. tuberculosis* as well as in virulent *M. bovis.* The esat-6 gene could not be demonstrated in any of the eight substrains of *M. bovis* BCG tested by these techniques. Two gene deletions that distinguish *M. bovis* BCG from virulent *M. bovis* have thus now been demonstrated. Deletion of mpb64 affects four of the eight substrains tested; deletion of esat-6 affects all of them. The reaction of HYB76-8 at 26 kDa with four of the BCG substrains was demonstrated to result from crossreactivity with MPB64. HYB76-8 was also shown to cross-react with the A, B, and C components of the antigen 85 complex and MPT51.—Authors' Abstract

Johnson, P. D. R., Veitch, M. G. K., Leslie, D. E., Flood, P. E. and Hayman, J. A. The emergence of *Mycobacterium ulcerans* infection near Melbourne (Australia). Med. J. Aust. **164** (1996) 76–78.

Objective: To document the emergence of new foci of *Mycobacterium ulcerans* infection (Bairnsdale ulcer) in Victoria.

Methods: From data kept by one of us (JAH) and records from the Mycobacterium Reference Laboratory, Fairfield Hospital, we reviewed cases of *M. ulcerans* infection in Victoria between 1980 and 1995, and identified those apparently acquired outside the east Gippsland endemic region. A case was defined as a person with a lesion suggestive of *M. ulcerans* infection, from which the organism had been cultured or, in the absence of culture information, from which a histological specimen characteristic of *M. ulcerans* infection had been obtained.

Results: We identified 45 people who appeared to have acquired their infections in Victoria but outside the east Gippsland region. A new focus appeared on the northern shores of Western Port, near Melbourne, in 1982, and there was a dramatic increase in cases between 1991 and 1994 associated with foci on Phillip Island, and in the Frankston-Langwarrin area of outer suburban Melbourne. Single cases came from Crib Point, Narre Warren and Bendigo.

Conclusions: There have been at least three new foci of *M. ulcerans* infection within 80 km of Melbourne since 1982. Victorian clinicians should consider the possibility of Bairnsdale ulcer when dealing with unusual skin lesions.—Authors' Abstract

Kingston, A. E., Hicks, C. A., Colston, M. J. and Billingham, M. E. J. A 71-kDa heat shock protein (hsp) from *Mycobacterium tuberculosis* has modulatory effects on experimental rat arthritis. Clin. Exp. Immunol. **103** (1996) 77–82.

The effects of a mycobacterial 71-kDa heat shock protein (hsp) antigen have been investigated for its ability to modulate arthritis in rats. Subcutaneous injection (base of tail) of increasing amounts of hsp71 from *Mycobacterium tuberculosis* (MTB) produced dose-dependent differential inhibitory effects on induction of arthritis by MTB and CP20961 in rats. As little as 1 μg of the hsp71 produced a reduction in MTB arthritis; whereas complete protection was observed when 50 μg were administered. When 71-kDa-treated rats were challenged with CP20961, all developed reduced symptoms of arthritis compared with control rats, but in this model no complete protection was observed over the dose range studied. The effects of 71-kDa pretreatment on collagen II arthritis were not significant, but in general symptoms of arthritis were milder than in the control group. The same pattern of results was observed previously when hsp65 was used in the different models. These results show that the modulatory effects of hsp on adjuvant arthritis are not restricted to the hsp65 series, but are also mediated by a member of the hsp 70 family.—Authors' Abstract

Kirschner, P., Rosenau, J., Springer, B., Teschner, K., Feldmann, K. and Bottger, E. C. Diagnosis of mycobacterial infections by nucleic acid amplification: 18-month prospective study. J. Clin. Microbiol. **34** (1996) 304–312.

We have investigated the use of DNA amplification by polymerase chain reaction (PCR) for the detection of mycobacteria in clinical specimens, with the gene encoding the 16S rRNA as a target. Following generic amplification of mycobacterial nucleic acids, screening was done with genus-specific probe; this was followed by species differentiation by use of highly discriminating probes or nucleic acid sequencing. In a prospective 18-month evaluation, criteria to select specimens for PCR analysis were defined. Of a total of 8272 specimens received, 729 samples satisfied the criteria and were subjected to DNA amplification. Clinical specimens included material from the respiratory tract (sputa and bronchial washings), aspirates, biopsies, and various body fluids (cerebrospinal, pleural, peritoneal, and gastric fluids). After resolution of discrepant results, the sensitivity of the PCR assay was 84.5%, the specificity was 99.5%, the positive predictive value was 97.6%, and the negative predictive value was 96.4%. The sensitivity and negative predictive value of culture (with a combination of broth and solid media) were 77.5% and 94.8%, respectively. In conclusion, this PCR assay provides an efficient strategy to detect and identify multiple mycobacterial species and performs well in comparison with culture.—Authors' Abstract

Laochumroonvorapong, P., Paul, S., Elkon, K. B. and Kaplan, G. H_2O_2 induces monocyte apoptosis and reduces viability of *Mycobacterium avium-M. intracellulare* within cultured human monocytes. Infect. Immun. **64** (1996) 452–459.

Mycobacterium avium-M. intracellulare, an intracellular parasite of mononuclear phagocytes, rarely causes disease in immunocompetent individuals. In contrast, in human immunodeficiency virus type 1-infected patients, *M. avium-M. intracellulare* can infect almost every tissue and organ. This suggests that immunocompetent individuals have a protective mechanism to control or prevent the infection. How mycobacteria may be killed by the host immune response is unclear. We have recently reported that induction of apoptosis of *M. bovis* BCG-infected macrophages with ATP(4-) was associated with killing of the intracellular mycobacteria. In the present study, a long-term culture of *M. avium-M. intracellulare*-infected monocytes was used to further evaluate the interaction between *M. avium-M. intracellulare* and primary human monocytes. In our system, *M. avium-M. intracellulare* parasitized the human monocytes and appeared to replicate slowly over 14 days within the host cells. To examine the role of apoptotic mechanisms in survival or death of intracellular mycobacteria, *M. avium-M. intracellulare*-infected human monocytes were treated with a monoclonal antibody to Fas receptor (APO-1/CD95) or with various concentrations of H_2O_2. Although both of these exogenous agents induced monocyte apoptosis, optimal killing (65% reduction in CFU) of intracellular *M. avium-M. intracellulare* was observed only when *M. avium-M. intracellulare*-infected cells were treated with

10 mM H_2O_2. Fas-induced apoptosis did not affect *M. avium-M. intracellulare* viability. Our results suggest that not all stimuli of monocyte apoptosis induce killing of intracellular *M. avium-M. intracellulare*. Since release of H_2O_2 following phagocytosis of mycobacteria has been documented, H_2O_2-induced apoptotic death of *M. avium-M. intracellulare*-infected monocytes and its association with killing of the intracellular bacilli may be a physiological mechanism of host defense against *M. avium-M. intracellulare*.—Authors' Abstract

Lee, L. T., Chien, C. J., Lee, W. C., Luh, K. T., Hsieh, W. C. and Lin, R. S. Age-period-cohort analysis of pulmonary tuberculosis mortality in Taiwan: 1961 to 1990. J. Formos. Med. Assoc. **93** (1994) 657–662.

The specific aim of this study was to examine the effects of age, calendar period of death, and birth cohort in pulmonary tuberculosis (TB) mortality in Taiwan during the period 1961 to 1990. A log-linear Poisson regression model modified from the method of Osmond and Gardner was used and 79, 881 deaths (58,025 males and 21, 856 females) were included in the analysis. Birth cohort is the most significant predictor of pulmonary TB mortality according to the model. The earliest birth cohort from 1991 had a pulmonary TB mortality 17, 327 and 6186 times those born from 1986 for males and females, respectively. There was also a significant age effect. The youngest age group of zero to 4 years had a pulmonary TB mortality 7.10 and 5.87 times those for the age group of 5 to 9 years for males and females, respectively. The oldest age group of 70 to 74 years had a risk of pulmonary TB mortality 2.89 and 1.88 times those for the 5- to 9-year-age groups for males and females, respectively. Parameters of the period factor showed a decreasing pulmonary TB mortality from 1961 to 1990 that was less significant than those of age or cohort. In addition to the improvement in medical measures that influenced the effect of calender year on TB mortality, year of birth is an important determinant in the trend of TB mortality in Taiwan. The result shows that the major focus for TB mortality in Taiwan is in the age groups born between 1891 and 1921.—Authors' Abstract

Lee, R. E., Mikusova, K., Brennan, P. J. and Besra, G. S. Synthesis of the mycobacterial arabinose donor beta-D-arabinofuranosyl-1-monophosphoryldecaprenol, development of a basic arabinosyl-transferase assay, and identification of ethambutol as an arabinosyl transferase inhibitor. J. Am. Chem. Soc. **117** (1995) 11829–11832.

Mycobacterial diseases, such as tuberculosis and leprosy, are serious human pathogens, in which the cell-wall arabinans of arabinogalactan and lipoarabinomannan are essential components of the bacterial cell wall. The chemical synthesis of the key mycobacterial arabinose donor [1-C-14]-beta-D-arabinofuranosyl-1-monophosphoryldecaprenol 2 is described by an application of the phosphoramidite-phosphite triester methodology to form the beta-arabinofuranosyl, allylic phosphodiester. The synthesis uses a novel tert-butyl dimethylsilyl arabinofuranosyl protection strategy, which allows for a regioselective C-1 acid hydrolysis and final full deprotection with ammonium fluoride under mild conditions. A basic arabinosyl transfer assay was developed to study the incorporation of 2 into the cell-wall arabinans of mycobacteria. The incorporation was proportional with respect to both the concentration of membrane protein and the acceptor. The epimeric substrate [1-C-14]-alpha-D-arabinofuranosyl-1-monophosporylde-caprenol was inactive and noninhibitorial in this assay. The antimycobacterial drug ethambutol was found to be active, suggesting that its mode of action is as an inhibitor of arabinosyl transfer.—Authors' Abstract

Lowrie, D. B., Tascon, R. E. and Silva, C. L. Vaccination against tuberculosis. Int. Arch. Allergy Immunol. **108** (1995) 309–312.

Recent findings in mice have changed our perception of how protective immunity works in tuberculosis and hold promise for the rapid development of new vaccines. For example, we now know: (1) that a single mycobacterial protein antigen can be sufficient to generate powerful protective immunity, provided that it is presented to the immune system in the right way; (2) that the expression of protection depends on cy-

totoxic antigen-specific T cells; (3) that the identity of the antigen may be less important than the mode of presentation, and (4) that injection of DNA encoding the antigen (DNA vaccination) is a superior way of raising protective immunity compared to injection of the antigen itself. These advances are timely because there is an urgent need for a new vaccine against tuberculosis. There continue to be about 3 million deaths from tuberculosis every year worldwide and increasingly the causative bacteria are multidrug resistant.—Authors' Abstract

Mazurek, G. H., Reddy, V., Murphy, D. and Ansari, T. Detection of *Mycobacterium tuberculosis* in cerebrospinal fluid following immunomagnetic enrichment. J. Clin. Microbiol. **34** (1996) 450–453.

The detection of *Mycobacterium tuberculosis* by culture of cerebrospinal fluid (CSF) is unacceptably slow. Low numbers of organisms and the presence of reaction inhibitors may prevent detection of *M. tuberculosis* by PCR. We used immunomagnetic enrichment to accelerate and enhance the detection of mycobacteria in CSF after demonstrating the utility of the method with pure suspension. Growth was detected earlier in Bactec cultures of magnetically recovered mycobacteria than in untreated CSF (7 versus 15 days). We detected *M. tuberculosis* DNA by PCR in the immunomagnetically enriched sample but not in untreated CSF. PCR fingerprintings of the immunomagnetically recovered *M. tuberculosis* and of the isolate subsequently recovered by culture were identical.—Authors' Abstract

Morisaki, N., Kobayashi, H., Iwasaki, S., Furihata, K., Dabbs, E. R., Yazawa, K. and Mikami, Y. Structure determination of ribosylated rifampicin and its derivative: new inactivated metabolites of rifampicin by mycobacterial strains. J. Antibiot. **48** (1995) 1299–1303.

Rifampicin (1) was converted into two inactivated products RIP-Ma and RIP-Mb by *Mycobacterium smegmatis* DSM43756. MS, NMR and chromatographic analysis showed the compounds to be 3-formyl-23-[O-(alpha-D-ribofuranosyl)]rifamycin SV (6) and 23-[O-(alpha-D-ribofuranosyl)]rifampicin (7), respectively.—Authors' Abstract

Ortalomagne, A., Lemassu, A., Laneelle, M. A., Bardou, F., Silve, G., Gounon, P., Marchal, G. and Daffe, M. Identification of the surface-exposed lipids on the cell envelopes of *Mycobacterium tuberculosis* and other mycobacterial species. J. Bacteriol. **178** (1996) 456–461.

The surface-exposed lipids of *Mycobacterium tuberculosis. M. avium, M. kansasii, M. gastri, M. smegmatis, and M. aurum* were isolated by gentle mechanical treatment of cells with glass beads. Analysis of the exposed lipids demonstrated a selective location of classes of ubiquitous lipids on the surfaces of mycobacteria. While phosphatidylethanolamine and phosphatidylinositol mannosides were exposed in all the species examined, dimycoloyl trehalose ("cord factor") was identified in the surface components of M. aurum only, Furthermore, monomycoloyl trehaloses and triacylglycerides were identified in the surface-exposed lipids of *M. avium* and *M. smegmatis* but not in those of the other mycobacterial species examined, The species- and type-species-specific lipids were present on the mycobacterial cell surface: phenolic glycolipids, dimycocerosates of phthiocerols, and lipooligosaccharides were identified in the surface-exposed materials of *M. tuberculosis* (Canetti), *M. kansasii,* and *M. gastri*; whereas glycopeptidolipids were identified in the outermost lipid constituents of *M. avium* and *M. smegmatis.* This difference in the surface exposure of lipids of various mycobacterial species may reflect differences in their cell envelope organizations, Brief treatments of *M. tuberculosis* with Tween 80 prior to the use of glass beads led to erosion of regions of the capsule to expose gradually both cord factor and other lipids on the cell surface of the tubercle bacillus, demonstrating that the latter lipids are buried more deeply in the cell envelope and leading to the proposal of a scheme for the location of the capsular lipids of the tubercle bacillus.—Authors' Abstract

Peterson, P. K., Gekker, G., Bornemann, M., Chatterjee, D. and Chao, C. C. Thalidomide inhibits lipoarabinomannan-induced upregulation of human immuno-

deficiency virus expression. Antimicrob. Agents Chemother. **39** (1995) 2807–2809.

Mycobacterium tuberculosis accelerates the progression of human immunodeficiency virus type 1 (HIV-1) infection. The results of this study, which show that thalidomide inhibits the upregulation of HIV-1 expression in U1 cells stimulated with mycobacterial lipoarabinomannans, support the rationale behind conducting controlled trials of this immunomodulatory agent with patients dually infected with HIV-1 and *M. tuberculosis.*—Authors' Abstract

Portillo-Gomez, L., Nair, J., Rouse, D. A. and Morris, S. L. The absence of genetic markers for streptomycin and rifampicin resistance in *Mycobacterium avium* complex strains. J. Antimicrob. Chemother. **36** (1995) 1049–1053.

Mycobacterium avium, M. intracellulare complex (MAC) bacilli are an important cause of bacteremia in AIDS patients but treatment is complicated by their resistance to the usual antimycobacterial agents. In this study of 20 strains of MAC none was found to have the mutations associated with resistance to rifampicin and streptomycin in *M. tuberculosis,* suggesting that MAC have unique mechanisms for resistance to these agents.—Authors' Abstract

Reddy, V. M., Nadadhur, G., Daneluzzi, D., O'Sullivan, J. F. and Gangadharam, P. R. J. Antituberculosis activities of clofazimine and its new analogs B4154 and B4157. Antimicrob. Agents Chemother. **40** (1996) 633–636.

In our efforts to develop new drugs for the treatment of tuberculosis, especially that caused by multidrug-resistant strains, we investigated clofazimine (CFM) and two of its analogs, B4154 and B4157, for their antituberculosis activities. Twenty *Mycobacterium tuberculosis* strains were tested, including 16 drug-resistant strains (strains resistant to one or more antituberculosis drugs), for their susceptibilities to these three agents. All of the strains were found to be susceptible to B4154 and B4157, and one strain showed moderate resistance to CFM. The MICs of B4154, B4157, and CFM at which 90% of strains were inhibited were 0.25, 0.12, and ≤ 1.0 μg/ml, respectively. The intracellular activities of CFM and B4157 were superior to that of B4154. The chemotherapeutic activities of the three compounds were evaluated in C57BL/6 mice. At a dose of 20 mg/kg of body weight, the activity of CFM was slightly superior to that of B4157; however, both compounds prevented mortality and caused a significant reduction in the numbers of CFU in the lungs and spleens. The animals treated with B4157 showed less pigmentation than animals treated with CFM. The chemotherapeutic activity of CFM was comparable to those of rifampin and isoniazid. Complete susceptibility of multidrug-resistant strains to CFM and B4157 and the therapeutic efficacies of these compounds against mouse tuberculosis make these drugs attractive agents for the treatment of drug-resistant tuberculosis.—Authors' Abstract

Rydberg, J., Miorner, H., Chandramuki, A. and Lantz, M. Assessment of a possible imbalance between tumor necrosis factor (TNF) and soluble TNF receptor forms in tuberculous infection of the central nervous system. J. Infect. Dis. **172** (1995) 301–304.

Distributions of tumour necrosis factor (TNF) and its soluble receptor forms, R55-BP and R75-BP, were analyzed in the cerebrospinal fluid of 71 patients with severe acute or chronic central nervous system infections. Tuberculous infections were associated with high ratios of R55-BP and R75-BP to TNF, 27.2 and 28.0, respectively, suggesting a small biologically active fraction of TNF. The opposite was found in subjects with acute bacterial meningitis: they had large functions of biologically active TNF and thus low ratios of R55-BP and R75-BP to TNF, 3.7 and 4.0, respectively. It is hypothesized that chronic infectious diseases, such as tuberculous infections, may be associated with inadequate production of TNF and a concomitant relative increase of soluble TNF receptors, which may prolong the disease.—Authors' Abstract

Walker, G. T., Nadeau, J. G., Linn, C. P., Devlin, R. F. and Dandliker, W. B. Strand displacement amplification (SDA) and

transient state fluorescence polarization detection of *Mycobacterium tuberculosis* DNA. Clin. Chem. **42** (1996) 9–13.

Strand displacement amplification (SDA) is an isothermal, *in vitro* method of amplifying a DNA sequence for diagnostic purposes. We have combined SDA with fluorescence polarization detection in a closed, homogeneous format. A fluorescently labeled oligodeoxynucleotide detect-or probe hybridizes to the amplification product that increases in concentration during SDA. The single- to double-stranded conversion of the probe is accompanied by an increase in fluorescence polarization values, which can be measured in real-time without physical manipulation of the sample. The probe was labeled with the near-infrared dye La Jolla blue, and fluorescence polarization was measured on a transient-state fluorometer. We have applied this homogeneous SDA/detection system to a target DNA sequence specific for *Mycobacterium tuberculosis* DNA.—Authors' Abstract

Watanabe, M., Honda, I., Kawajiri, K., Niinuma, S., Kudoh, S. and Minnikin, D. E. Distribution of antibody titres against phenolic glycolipids from *Mycobacterium tuberculosis* in the sera from tuberculosis patients and health controls. Res. Microbiol. **146** (1995) 791–797.

Sera from tuberculosis (TB) patients and healthy controls were tested by ELISA for their antibody titers against the two major phenolic glycolipids (PGLs) of *Mycobacterium tuberculosis*, PGL-tb0 (a 1:3 mixture of PGL-tb1 and its analog whose phthiocerol moiety is phenolphthiotriol A) and PGL-tbK. Both PGL-tbs were shown to be specific to *M. tuberculosis*, and the profiles of serum anti-PGL-tbK titers revealed that PGL-tbK, like PGL-tb1, was fairly widely distributed among strains of *M. tuberculosis*. Even when these two PGL-tbs were used, however, the rate of ELISA-positives was not very high among TB patients, which is probably explained by the nature of the disease. Moreover, a considerable number of sera from healthy controls, especially from younger age groups, had high anti-PGL-tb titers, which implies that environmental exposure to *M. tuberculosis* is much higher than has been estimated from the actual TB cases. The ELISA system using these species-specific PGL-tb antigens may be useful for the survey of TB infection, since it gives more direct information on TB infection than the PPD skin test.—Authors' Abstract

Yajko, D. M., Sanders, C. A., Madej, J. J., Cawthon, V. L. and Hadley, W. K. *In vitro* activities of rifabutin, azithromycin, ciprofloxacin, clarithromycin, clofazimine, ethambutol, and amikacin in combinations of two, three and four drugs against *Mycobacterium avium*. Antimicrob. Agents Chemother. **40** (1996) 743–749.

Multidrug therapy is recommended for treatment of *Mycobacterium avium* complex (MAC) bacteremia in patients with AIDS. Azithromycin, clarithromycin, rifabutin, ciprofloxacin, ethambutol, clofazimine, and amikacin have all been suggested for use in treating MAC bacteremia, but the most active combinations of these drugs have not been identified, nor has the minimum number of drugs needed for effective therapy been determined. To address the former, the *in vitro* bactericidal activities of all two-, three-, and four-drug combinations of these seven agents was determined by using 10 blood-derived strains of MAC isolated from patients with AIDS. The activities of the 132 drug combinations were compared by statistical analysis of survival means (analysis of variance) and further evaluated by determining the percentage of strains considered susceptible to each combination. When susceptibility was defined as a decrease in CFU of ≥ 2 $\log_{10}$, no two- or three-drug combination and only two four-drug combinations were active against all 10 MAC strains. When a less stringent definition was applied (≥ 1 $\log_{10}$ decrease in CFU), 1 two-drug combination, 9 three-drug combinations, and 31 four-drug combinations showed activity against all 10 strains. Eighteen selected drug combinations were also tested for intracellular activity in MAC-infected J774 cells. Combinations which contained amikacin as a component were considerably less active against intracellular MAC organisms than against organisms in broth. The opposite result was obtained for the combination of clarithromycin plus clofazimine.—Authors' Abstract