CURRENT LITERATURE

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

General and Historical

Awofeso, N. Effect of socio-cultural beliefs on patients' perception of leprosy—the general factor. Trop. Geogr. Med. **47** (1995) 175–178.

A study to determine the gender factor in the effect of socio-cultural beliefs on patients' perceptions of leprosy was conducted with the participation of 293 leprosy patients in Kaduna State, Nigeria. Results indicate that, contrary to popular belief, the male model is not the single interpretative model for leprosy as far as socio-cultural aspects are concerned. These differences are analyzed and appropriate suggestions made.—Author's Abstract

Lockwood, D. N. J. and Saunderson, P. R. Harnessing the strengths of the leprosy programme to control tuberculosis. Br. Med. J. **311** (1995) 862–863.

Tuberculosis remains a leading cause of death in Ethiopia, but there is no effective national tuberculosis control program. By contrast, the leprosy control program has been very successful, with a 10-fold reduction in the number of leprosy cases requiring antibacterial treatment, although patients with nerve damage require continuing care. The paradox of rising numbers of tuberculosis cases and declining numbers of leprosy cases may be solved by joint leprosy-tuberculosis clinics. The strengths of leprosy fieldworkers in control management, case holding, and compliance can be harnessed in developing an effective tuberculosis control program. Implementing a joint program in Ethiopia may be beneficial not only for tuberculosis patients but also for leprosy patients, who are thus brought closer to general medical services.—Authors' Abstract

McDougall, A. C. Training in leprosy: does the current strategy need revision? (Editorial) Lepr. Rev. **66** (1995) 89–95.

The editorialist argues that the integration of leprosy with general health services will probably be an important option for the future, using the primary or peripheral health care system with supervision at the district level. For this integration to take place, large numbers of health care workers will need to be trained and provided with appropriate teaching and learning material, including the use of local languages. The strategy for training would need revision "without delay" to ensure that sufficient trainers were available, and the source of the trainers needs to be discussed. An appendix provides a useful list of 14 previously used training centers throughout the world and the contents of their courses.—C. A. Brown (Trop. Dis. Bull.)

Raju, M. and Kopparty, S. N. M. Impact of knowledge of leprosy on the attitude towards leprosy patients; a community study. Indian J. Lepr. **67** (1995) 259–272.

NLEP, through its survey-education-treatment (SET) pattern, attempts to educate the community members about the scientific facts of leprosy with the view to improve their knowledge leading to a more positive attitude toward the leprosy-afflicted. This paper explores the impact of knowledge on the attitudes of 1199 community members drawn from two Indian states, Andhra Pradesh and Orissa, toward leprosy. The results show that, overall, a high knowledge level did not necessarily generate positive attitudes. There was a general negative attitude despite 35% to 50% of the respondents having a high knowledge level. There were, however, situations in which a high level of knowledge helps to have positive attitudes. These situations differ in the two states studied.—Authors' Abstract

Chemotherapy

Dhople, A. M. and Ibanez, M. A. *In vitro* activity of levofloxacin, singly and in combination with rifamycin analogs, against *Mycobacterium leprae.* Antimicrob. Agents Chemother. **39** (1995) 2116–2119.

The *in vitro* susceptibility of *Mycobacterium leprae* to levofloxacin was studied by using two biochemical parameters to measure the metabolic activity of the organism. Levofloxacin consistently exhibited twofold greater bactericidal activity than ofloxacin, with the MIC being 0.75 μg/ml. When combined with one of the three rifamycin analogs, synergism was obtained with KRM-1648 and rifabutin but not with rifampin.—Authors' Abstract

Faruque, E., Hoque, A. K. M. A., Ahmed, J., Ahmed, R. and Ali, A. [Paucibacillary leprosy patients treated with multidrug therapy, four years experience (1988 to 1991) in Bangladesh.] Jpn. J. Lepr. **63** (1994).

Two-hundred-fifty paucibacillary (PB) leprosy patients were treated with WHO-recommended multidrug therapy (MDT) and followed up for 4 years. The paucibacillary MDT regimen (PBR) was well accepted by the patients. Clinical regression was attained in 60% of patients after six doses of PBR. Reversal reaction occurred in 14% of the cases and relapse was found in 1.6% of cases 18–24 months after completing the treatment. The incidence of reversal reaction was high in patients with more than two thickened nerve trunks associated with more than five patches.—Authors' Abstract

Ji, B., Jamet, P., Perani, E. G., Bobin, P. and Grosset, J. H. Powerful bactericidal activities of clarithromycin and minocycline against *Mycobacterium leprae* in lepromatous leprosy. J. Infect. Dis. **168** (1993) 188–190.

Thirty-six patients from Mali with newly diagnosed lepromatous leprosy were allocated randomly to three groups and treated for 56 days with minocycline (100 mg daily), clarithromycin (500 mg daily), or clarithromycin (500 mg) plus minocycline (100 mg daily). All groups had rapid and remarkable clinical improvement and significant decline of the bacterial and morphological indices in skin smears during treatment. More than 99% and >99.9% of the viable *Mycobacterium leprae* had been killed by 28 and 56 days of treatment, respectively, as measured by inoculation of organisms recovered from skin samples, taken before and during treatment, into the foot pads of immunocompetent and nude mice. Clinical improvement and bactericidal activity did not differ significantly among the three groups. Adverse reactions were rare and mild, and no laboratory abnormality was detected during the trial. Both clarithromycin and minocycline displayed powerful bactericidal activities against *M. leprae* in leprosy patients and may be considered important components of new multidrug regimens for the treatment of multibacillary leprosy.—Authors' Abstract

Kar, P. K., Arora, P. N., Ramasastry, C. V. and Dhaka, R. A. A clinicopathological study of multidrug therapy in borderline tuberculoid leprosy. J. Indian Med. Assoc. **92** (1994) 336–337.

The standard WHO multidrug regimen (rifampicin 600 mg monthly, dapsone 100 mg daily, for 6 months) provided very inadequate chemotherapy for a group of 50 patients in Pune, India, with borderline tuberculoid leprosy, when assessed immediately after completion of the drug regimen; active lesions were found in 60% of the patients.—Authors' Abstract

Liu, C., *et al.* [Monitoring 453 persons for 4 years after MDT.] China Lepr. J. **11** (1995) 61–63. (in Chinese)

The results of four-year monitoring after completing MDT in 453 cases of multibacillary leprosy in Wenshan Prefecture, Yunnan, show that clinical conditions and bacterial indexes continued improving and decreasing, respectively, and there was no relapse.—Authors' English Abstract

Mor, N., Simon, S., Mezo, N. and Heifets, L. Comparison of activities of rifapentine and rifampin against *Mycobacterium tuberculosis* residing in human macrophages. Antimicrob. Agents Chemother. **39** (1995) 2073–2077.

The activities of rifapentine and rifampin against *Mycobacterium tuberculosis* residing in human monocyte-derived macrophages were determined. The MICs and MBCs of rifapentine for intracellular bacteria were two- to fourfold lower than those of rifampin. For extracellular bacteria, this difference was less noticeable. Nevertheless, the more favorable pharmacokinetics of rifapentine over rifampin was addressed in other experimental models. These models showed substantial differences after short pulsed exposures of the infected macrophages to the drugs and when the infected macrophages were exposed to changing drug concentrations that limited the pharmacokinetic curves observed in blood. Once-a-week exposures to rifapentine concentrations equivalent to those attained in blood after one 600-mg dose resulted during the first week in a dramatic decline in the number of bacteria, and this decline was maintained at a minimal level for a period of 4 weeks. The results of this study have shown the suitability of rifapentine for intermittent-treatment regimens. The prolonged effect of rifapentine found in this study may be associated with high ratios of intracellular accumulation, which were four- to fivefold higher than those found for rifampin. Further studies on the intracellular distribution of rifamycins and on the sites of actual interaction between the drugs and bacteria residing in macrophages are necessary.—Authors' Abstract

Ohenga, J. W., K'Omollo, J. W. and Ochieng, J. The effect of leprosy management on leprosy complications at Alupe Hospital, Busia, Kenya. Afr. J. Health Sci. **1** (1994) 88–92.

A retrospective study of leprosy complications (such as dryness, ulcers, contractures) among new cases recruited for multiple drug therapy (MDT) at Alupe Leprosy Hospital between January 1986 and January 1992 was carried out to determine their prevalence, incidence and their relationship with disease classification. Out of 154 cases studied, the majority fell in the borderline tuberculoid (BT) class (47%). The total follow-up period was 186 person-years giving an overall incidence rate of reactions of 20/100 person-years. Overall point prevalences of complications for both males and females were highest in the extremities and ranged between 10% and 46%, while the overall period prevalence ranged between 14% and 73% per year. There was a significant number of incident cases of complications during the follow-up period and comparison of different complications with different disease classes had odds ratios of around unity, showing that MDT alone as an intervention measure plays no significant role in the onset or progress of leprosy complications. It is observed that BT and borderline lepromatous (BL) leprosy patients are at the highest risk of developing leprosy complications.—Authors' Abstract

Sahai, J., Garber, G., Gallicano, K., Oliveras, L. and Cameron, D. W. Effects of the antacids in didanosine tablets on dapsone pharmacokinetics. Ann. Intern. Med. **123** (1995) 584–587.

Objective: To investigate 1) the effects of the magnesium-aluminum antacids in didanosine tablets on dapsone absorption in healthy volunteers and 2) the effects of the antacid formulation of active didanosine tablets on dapsone pharmacokinetics in patients seropositive for human immunodeficiency virus (HIV).

Design: Two separate, randomized, two-period, two-treatment, crossover studies with a 21-day washout period between treatments.

Setting: Clinicial investigation unit of a university hospital.

Participants: Six healthy men and six HIV-positive men.

Measurements: Serial dapsone plasma concentrations were measured when dapsone, 100 mg, was administered alone and with either the third of four doses of didanosine placebo tablets (group 1) or the 27th of 28 doses of active didanosine tablets (group 2). In each study, pharmacokinetic variables were determined using noncompartmental methods and compared by analysis of variance.

Results: When dapsone was administered alone, pharmacokinetic variables did not significantly differ from those with dapsone given in either combination ($p > 0.10$ for all comparisons).

Conclusions: Didanosine, antacids, and other excipients in the currently used didanosine chewable tablets did not significantly affect plasma concentrations of dapsone.—Authors' Abstract

Save, M. P., Shetty, V. P. and Antia, N. H. Intracellular localization of dapsone and rifampicin in skin and nerve of multidrug treated leprosy patients. Indian J. Lepr. **67** (1995) 273–284.

Intracellular localization of antileprosy drugs dapsone (DDS) and rifamp in (RFP) was carried out on skin and nerve lesions obtained from multidrug treated, multibacillary (BL-LL) and paucibacillary (BT-TT) cases of leprosy using immunocytochemical techniques. Intracellular localization of the above drugs especially in macrophages and Schwann cells was aimed by using rabbit raised anti-DDS and -RFP polyclonal antibody in an indirect peroxidase assay. Our study records both intra- and extracellular staining with anti-DDS and -RFP antibodies in the skin as well as nerve lesions of MB and PB cases treated with MDT. All the nerves under investigation had moderate to severe pathology and, hence, free diffusion of the drug could be attributed to the broken barrier. Basal lamina around the Schwann cell did not seem to form a barrier. It was also noted that the drug (metabolite) persisted over a long period of time.—Authors' Abstract

Yu, X. [Monitoring after MDT in 1076 cases of leprosy.] China Lepr. J. **11** (1995) 76–77. (in Chinese)

In the period 1982 to 1991, 927 new cases of leprosy and 149 relapsed ones who had been cured with DDS alone, including 619 MB and 457 PB, took MDT until Bl had become negative and clinicial symptoms disappeared, and then continued to take DDS plus RMP for 1 year followed by clinical and bacteriological examinations once a year; 762 cases have been monitored for 3 years on an average or 2284.5 PYS after the treatment and only one BT case relapsed.—Author's English Abstract

Yu, I., *et al.* [Surveillance for 8 years after completion of MB-MDT.] China Lepr. J. **11** (1995) 59–61. (in Chinese)

Thirty-three MB leprosy patients after completion of MDT have been monitored for 8 years; there was no relapse and Bl in them all become negative within 5 years of the monitoring.—Authors' English Abstract

Clinical Sciences

Courtright, P. and Lewallen, S. Current concepts in the surgical management of lagophthalmos in leprosy. Lepr. Rev. **66** (1995) 220–223.

Existing data suggest that, at a minimum, 2% of paucibacillary patients and 5% of multibacillary patients have lagophthalmos; at least 290,000 people worldwide have leprosy-related lagophthalmos. Surgical intervention is the only method for correcting lagophthalmos; effectiveness of the different procedures commonly used has not been measured. Results from a survey of eye care providers revealed that surgeons in Asia used a wide range of different techniques for the correction of lagophthalmos while almost all of the surgeons in Africa used tarsorrhaphy. There is a need to evaluate surgical outcome of these techniques and to develop guidelines to assist in increasing the number of surgeries for lagophthalmos in leprosy patients.—Authors' Summary

Courtright, P., Lewallen, S., Li, H.-Y., Hu, L.-F. and Yang, J.-W. Lagophthalmos in a multibacillary population under multidrug therapy in the People's Republic of China. Lepr. Rev. **66** (1995) 214–219.

Lagophthalmos may be the most common potentially blinding ocular condition

in leprosy. The magnitude of the problem among multibacillary patients has not been determined. We sought to ascertain the magnitude of lagophthalmos in a multibacillary leprosy patient population under multidrug therapy (MDT) (both newly diagnosed and with a prior history of dapsone monotherapy) in China and assess factors associated with its presence. In a survey of 640 multibacillary patients 3.8% of the newly diagnosed patients and 10.2% of the patients with prior dapsone monotherapy had lagophthalmos. Corneal disease and vision loss were common in both groups. Poor compliance with MDT, duration between onset and diagnosis, and duration on dapsone monotherapy were associated with the presence of lagophthalmos.

Our findings suggest that there may be a threshold at which MDT must be maintained to prevent lagophthalmos. Early leprosy diagnosis and treatment would also lessen the incidence of lagophthalmos in these patients. The high proportion of lagophthalmos patients with corneal disease suggests that there has been inadequate eye care for these patients.—Authors' Summary

Dhar, S., Kaur, I., Dawn, G., Sehgal, S. and Kumar, B. Post-kala-azar dermal leishmaniasis mimicking leprosy: experience with 4 patients, with some unusual features in 1. Lepr. Rev. **66** (1995) 250–256.

We report on four cases of post-kala-azar dermal leishmaniasis (PKDL). History of kala-azar was available in all four patients. Slit-skin smears (SSS) for leishmania donovani (LD) bodies were negative in all four. In three patients hypopigmented lesions were present over the face. Papules and nodules over his lips, tongue, scrotum and dactylitis were some unusual features observed in one patient. Histopathological examination showed LD bodies in two patients; histopathology was nonspecific in the other two. All the patients were treated with sodium stibogluconate, 20 mg/kg/day. Infiltrated papules and nodules had subsided by 3 months, while hypopigmented macules took longer to improve. In three patients there had previously been a misdiagnosis as leprosy sufferers and they had been treated with antileprosy drugs. Clinical and histopathological differences between PKDL and leprosy are discussed.—Authors' Summary

Hagelskjaer, L. H. A fatal case of systemic strongyloidiasis and review of the literature. Eur. J. Clin. Microbiol. Infect. Dis. **13** (1994) 1069–1074.

The case of a 27-year-old woman from Bangladesh with lepromatous leprosy who developed systemic strongyloidiasis upon treatment with systemic steroids for an erythema nodosum leprosum reaction is presented. A literature review of systemic strongyloidiasis is also given.—Author's Abstract

Hieselaar, L. C. J. M., Hogeweg, M. and deVries, C. L. Corneal sensitivity in patients with leprosy and in controls. Br. J. Ophthalmol. **79** (1995) 993–995.

In a quantitative prospective study, the corneal sensation in patients with leprosy was compared with age matched controls. The patients with leprosy were classified as paucibacillary and multibacillary and were divided in three groups: (1) patients without clincially detectable eye pathology, (2) patients with lagophthalmos, (3) patients with signs of iridocyclitis. The corneal sensitivity was assessed with the Cochet and Bonnet aesthesiometer. There was a significant decrease in corneal sensitivity in multibacillary patients without clinically detectable eye pathology and in patients with lagophthalmos or iritis when compared with controls. A significant correlation between the loss of power of the orbicularis oculi muscle and the degree of corneal sensation loss could not be established. No significant decrease in corneal sensitivity was found in paucibacillary patients without eye pathology compared with the control group. The results of this study showed that loss of corneal sensation can occur while there is no clinically detectable eye pathology, at least in multibacillary patients. Regular checkups of the corneal sensation should, therefore, be part of the routine control of leprosy patients. Health education on eye care and early warning signs should be encouraged.—Authors' Abstract

Kolappan, C., Selvaraj, R., Khudoos, A., Appe Gowda, B. N., Datta, M. and Prabhakar, R. Repeatability of nerve thickness assessment in the clinical examina-

tion for leprosy. Lepr. Rev. **66** (1995) 224–228.

The assessment of the thickness of the superficial peripheral nerve trunks to document nerve involvement is an imporant aspect of clinical examination in case finding for leprosy, and is usually done by trained paramedical workers (PMWs). This assessment is subject to variability and has implications on the outcome of the survey. The present study proposes to quantify this variability. In this study, 242 individuals, consisting of 50 neuritic cases, 143 nonneuritic cases of leprosy and 49 normal controls, selected from the records of the trial of BCG prophylaxis in leprosy in South India, were examined by a doctor and PMWs. Repeatability of nerve thickness assessment for ulnar and popliteal nerves between the medical officer (MO) and the PMWs was quantified using Kappa statistics. The Kappa values for repeatability between the MO and the PMWs ranged from 0.45 to 0.54 and 0.52 to 0.69 for ulnar and popliteal nerves, respectively. The implications of the variability in nerve assessment are discussed.—Authors' Summary

Liu, X. [The causes of blindness and low vision in leprosy.] China Lepr. J. **11** (1995) 131–132. (in Chinese)

The causes of blindness and low vision have been analyzed among 457 active and cured leprosy patients in Taixing City, Jiangsu Province. The results showed that there were 13 bilateral (2.84%) and 31 unilateral (6.78%) blindness, 33 bilateral (7.22%) and 38 unilateral (8.32%) low vision, and they were all set off by corneal diseases, including keratitis, inflammation of the corenal stroma and corneal staphyloma, iritis, cataracts and glaucoma. Their incidence rate increased with the disease duration and age, especially in those with LL type.—Authors' English Abstract

Nanda Kishore, B. and Shetty, J. N. Bacterial clearance with WHO-recommended multidrug regimen for multibacillary leprosy. Indian J. Lepr. **67** (1995) 301–308.

Sixty multibacillary leprosy patients with an average initial bacterial index (BI) of 2.5 were followed up after they had completed the WHO-recommended multidrug therapy regularly until attaining bacteriological negativity. The minimum duration of treatment was 2 years as stipulated by WHO and the maximum duration for reaching negativity was 7 years (mean 4.25 years). The minimum time for the attainment of bacteriological negativity was 1 year and the maximum was 6.75 years (mean 3.75 years). The higher the initial BI the longer was the time taken for the attainment of bacteriological negativity. The average fall of BI per year was 0.67. Dapsone monotherapy received before the commencement of MDT, prednisolone received during therapy and the type of leprosy did not have any effect on the time taken for bacteriological clearance. There was no relapse during the period of observation (mean 2.83 years). The site to attain negativity last was the ear lobe in 95% of the cases.—Authors' Abstract

Nwosu, S. N. N. and Nwosu, M. C. Ocular findings in leprosy patients in Nigeria. E. Afr. Med. J. **71** (1994) 441–444.

An ophthalmic assessment of patients in 4 out of the 5 leprosy clinics in Anambra State, eastern Nigeria, showed that 63% had ocular disease and 43.5% had sight-threatening disorders. Most of the problems occurred in multibacillary leprosy patients. The blindness rate (8.7%) is nearly 10 times higher than that within the general population in the area. The causes of blindness were cataract, exposure keratopathy and uveitis. Some patients also had glaucoma and chorioretinal lesions. Eye health service within the leprosy control service in the area is nonexistent. The authors emphasize the importance of giving priority to blindness prevention in leprosy patients in the area.—Authors' Abstract

Tang, X. [Blindness among inpatients with leprosy.] China Lepr. J. **11** (1995) 129–131. (in Chinese)

In the period August 1994 to February 1995, 780 persons who have and had leprosy with the ages of 18–94 years were examined for blindness with less than 0.05 of corrected visual acuity in Guangzhou, Jieyang, Zhaoquing, Nanhai and Qingyuan, Guangdong Province. Ninety-six bilateral blindness patients were found, making up

19.62% of the cases. Causes were: cataracts in 36.2%, glaucoma in 30.4%, iritis in 25.5% and others in 7.9%.—Author's English Abstract

Yang, J., *et al.* [A survey of blindness and low vision in leprosy.] China Lepr. J. **11** (1995) 111–113. (in Chinese)

On the basis of WHO's standard, the visual disturbance of 2145 persons who have or had leprosy in Liangshan Prefecture and Panzhihua city had been examined, of which 20 persons were blind and 80 had diminution of vision bilaterally. The vision disability rate among them was 4.94%. The causes of the blindness and low vision mainly were leucoma and corneal ulcers which associate with the duration and type of leprosy, age, living and cultural levels, etc.—Authors' English Abstract

Yang, P., *et al.* [A survey of disability in 5106 leprosy patients.] China Lepr. J. **11** (1995) 133–136. (in Chinese)

According to WHO's standard, the disability and factors correlated with it have been investigated among 5106 persons who have and had leprosy during 1990 to 1991 in 15 counties of Shandong, including 4061 men and 1045 women with a mean age of 54.3 years. The result shows that 3002 persons have been disabled, accounting for 58.7%, including PB 3852 (55.09%) and MB 1254 (70.8%); 59.99% of the disabilities occured within 2 years after the disease, 40.91% before the treatment, and the longer the duration of the disease the more the disability. Manifestations of the disability mainly were claw hand, plantar ulcer and lagophthalmos, of which the causes mostly were lepra reaction and injury.—Authors' English Abstract

Yao, J. [Design and clinical use of a standard visual target card.] China Lepr. J. **11** (1995) 64–68. (in Chinese)

Existing vision test card used in the field is not standard and has some defects. According to the international design standard of visual target and correcting formula for vision test under condition of movable distance, a vision test card has been designed. With the card the visual target may be changed as the distance changes and conforms to internationally common visual acuity test chart based on the harmonious series system. It may be easily made and the test results in 75 cases showed that the card may be used for leprosy patients in the field.—Author's English Abstract

Immuno-Pathology

Adams, E., Basten, A., Prestridge, R. and Britton, W. J. T cell clones from a non-leprosy exposed subject recognize the *Mycobacterium leprae* 18-kD protein. Clin. Exper. Immunol. **102** (1995) 58–64.

Although *Mycobacterium leprae* shares many protein antigens with other mycobacterial species, there is a degree of specificity in the T-cell response to the organism. This is evident in the failure of cross-protection between mycobacterial species and the specific unresponsiveness to *M. leprae* in lepromatous leprosy patients. The antigenic basis of this specificity is unresolved, but the *M. leprae* 18-kDa protein is one candidate because of its restricted distribution and the isolation of *M. leprae*-specific T-cell clones reactive with the protein from *M. leprae*-vaccinated subjects. In the course of analyzing the human T-cell repertoire to mycobacteria we have isolated further CD4+ T-cell clones reactive with this protein from a subject who had never been exposed to *M. leprae*. These clones did not respond to other mycobacteria, including *M. tuberculosis* and *M. bovis* (BCG). In addition, they were unreactive with the *M. tuberculosis* 16-kDa protein which has recently been shown to have limited amino acid identity with the *M. leprae* 18-kDa protein. Both clones reacted with peptide 38–50 from the *M. leprae* 18-kDa protein, the T-cell response to which is restricted by HLA-DR4. Although homologues for the gene encoding the *M. leprae* 18-kDa antigen

have been identified in *M. avium* and *M. intracellulare,* the clones failed to respond to preparations of *M. avium.* Both clones secreted interferon-gamma and tumor necrosis factor-beta and were cytolytic against autologous targets pulsed with peptide 38–50 or the 18-kDa protein. The nature of the antigen which stimulates this apparently "*M. leprae*-specific" response is unknown. Nevertheless the recognition of the 18-kDa protein by individuals not exposed to leprosy indicates that this protein may not be suitable as a reagent to distinguish between infection with *M. leprae* and other pathogenic mycobacteria.—Authors' Abstract

Desikan, P., Parkash, O. and Narang, P. Role of antineural antibodies in perpetuation of a pre-existing peripheral nerve damage in leprosy. Indian J. Lepr. **67** (1995) 293–300.

This study was carried out in order to find out whether antineural antibodies had a role to play in perpetuating pre-existing nerve damage in leprosy. Indirect ELISA was carried out on sera from 20 leprosy patients and five normal controls using antigen prepared from peripheral nerves of a cured, bacteriologically negative leprosy patient. None of the patients had significant levels of IgG antibodies; whereas eight of them (40%) had significant levels of IgM antibodies. However, there was no correlation with duration of disease, treatment received, nerve enlargement or active neuritis. The nature of these antibodies is discussed.—Authors' Abstract

Misra, N., Murtaza, A., Walker, B., Narayan, N. P. S., Misra, R. S., Ramesh, V., Singh, S., Colston, M. J. and Nath, I. Cytokine profile of circulating T cells of leprosy patients reflects both indiscriminate and polarized T-helper subsets: T-helper phenotype is stable and uninfluenced by related antigens of *Mycobacterium leprae.* Immunology **86** (1995) 97–103.

Cytokine profiles of circulating mononuclear cells were studied with the aim of delineating T-cell subsets in leprosy patients with active disease. Using reverse transcriptase-polymerase chain reaction (RT-PCR) for cytokine mRNA and enzyme-linked immunoassay (ELISA) for the secreted products, interferon-gamma (IFN-γ), interleukin-4 (IL-4), IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) were studied. Three antigens, native *Mycobacterium leprae,* a recombinant antigen LSR/A15 of *M. leprae* and peptide 624 spanning 58-77 amino acids of the latter, were used to induce cytokine expression and release. Half of the subjects, irrespective of the clinical type or antigen used, showed a mixed T-helper type 0 (Th0)-like cytokine pattern, with evidence of the concomitant presence of IFN-γ and IL-4. The remainder showed a polarized pattern based on the type of leprosy. Lepromatous patients with disseminated disease had Th2-type cytokines, with IL-4 but not IFN-γ. In contrast, tuberculoid leprosy patients with localized disease showed a Th1-like profile, with the presence of IFN-γ but not IL-4. Of interest was the stability of the Th phenotype for *M. leprae*-related antigens. Both the recombinant and the peptide antigens induced the same phenotype as the natural *M. leprae* bacillus in all except four of 45 leprosy patients.—Authors' Abstract

Ochieng, N. A., Bwire, M. S., Orege, P. A., Nyawalo, J. O. and K'Omollo, J. W. Autoantibodies in lepromatous leprosy. Afr. J. Health Sci. **1** (1994) 79–83.

Lepromatous leprosy patients often develop erythema nodosum leprosum (ENL) reactions mainly during treatment of the disease. This study in Kenya sought to investigate the correlation between the prevalence of certain autoantibodies and ENL reactions in these patients. Serum samples were collected from 50 patients with lepromatous leprosy and a similar number of normal controls, and the prevalences of circulating rheumatoid factor and antinuclear and antismooth muscle antibodies were determined. The prevalence of autoantibodies was increased in lepromatous leprosy as compared with normal controls. The prevalences of these autoantibodies were affected differently by the ENL reactions. In lepromatous leprosy with ENL there was a slight decrease in the prevalence of antinuclear and antismooth muscle antibodies; whereas there was a slight increase in the

prevalence of rheumatoid factor. The levels of serum immunoglobulins tended to decline in lepromatous patients with ENL compared with the uncomplicated lepromatous patients. The significance of these findings is discussed in relation to their possible role in the pathogenesis of ENL reactions. It is concluded that some of these autoantibodies and immunoglobulins may be utilized during ENL reactions in the formation of immune complexes.—Authors' Abstract

Roman, E., Harris, D. P., Jurcevic, S., Ivanyi, J. and Moreno, C. H-2-associated effects of flanking residues on the recognition of a permissive mycobacterial T-cell epitope. Immunology **86** (1995) 183–189.

Previously we have identified an immunodominant, eight-residue, epitope core sequence (TAAGNVNI) fromthe 19,000 MW protein of *Mycobacterium tuberculosis*, which is recognized in the context of multiple H-2 I-A molecules. In this study, the role of residues flanking this T-cell epitope core was examined, using a series of 20 mer analogue peptides in which the native flanking residues were progressively replaced with L-alanine. Analogue peptides were tested for their capacity fo stimulate a CD4+ 19,000 MW protein-specific T-cell line, revealing that all but one N-terminal flanking residue could be replaced collectively by alanine without significant loss of stimulatory activity. However, clear H-2-associated differences in the requirement for flanking residues were demonstrated with peptide-specific T-cell hybridomas. In particular, H-2 (d)-derived hybridomas were much more stringent in their requirement for flanking residues than were H-2(b) hybridomas. All polyalanine-substituted peptides bound I-A(b) molecules, with affinities similar to the native unsubstituted peptide. In contrast, significantly reduced binding to I-A(d) was observed with several analogue peptides, although without a clear relationship to the degree of substitution. Furthermore, in H-2(b) mice, neither immunogenicity nor crossreactivity with the native peptide showed a clear inverse relationship with respect to the degree of alanine substitution. The results presented in this paper indicate that flanking residues can influence T-cell specificity and that these effects may be controlled by major histocompatibility complex (MHC) haplotype.—Authors' Abstract

Ruiz, P. and Geraldino, N. Peripheral gamma delta T-cell populations in HIV-infected individuals with mycobacterial infection. Cytometry **22** (1995) 211–216.

Previous studies have suggested that gamma delta T cells can be increased in HIV-1-seropositive individuals, although characterization of gamma delta T cell subtypes and correlation with clinicial status of these patients have not been performed. We investigated groups of adult HIV-seropositive persons to determine the prevalence of elevated levels of gamma delta T cells and whether any gamma delta T-cell subtypes were preferentially expressed. Since a large proportion of human gamma delta T cells appear to be reactive to proteins encoded by mycobacteria, we also examined our patients for the incidence of mycobacterial infection. Our results show that a significant number of HIV-positive patients have an elevated number of gamma delta T cells in their peripheral blood as compared to normal controls. HIV-seropositive patients with clinical or laboratory evidence of mycobacterial infection had statistically significant increases in the percentage and total numbers of gamma delta T cells over the HIV-positive persons without mycobacterial infection. An examination of the subtypes of gamma delta T cells revealed that certain subtypes such as V gamma 9+ and V delta 2+ T cells were preferentially elevated in the mycobacteria-positive patients. These results suggest that an increased number of gamma delta T cells in HIV-positive patients is most often seen in the setting of an opportunistic mycobacterial infection and that specific gamma delta T-cell subtypes are stimulated under these conditions. The role of these increased numbers of gamma delta T cells in HIV-associated disease is unclear but is likely a component of the response and degree of host resistance to this organism.—Authors' Abstract

Sasaki, K., Shibata, Y., Hashimoto, Y. and Iwasaki, S. Benzylphthalimides and phenethylphthalimides with thalidimoide-like activity on the production of tumor

necrosis factor alpha. Biol. Pharmaceut. Bull. **18** (1995) 1228–1233.

Benzylphthalimide analogs (P1Ps) and phenethylphthalimide analogs (P2Ps) have been found to exhibit thalidomide-like activity on the production of tumor necrosis factor (TNF)-alpha tetradecanoylphorbol-13-acetate (TPA). Structure-activity relationships are discussed on the basis of the TNF-alpha production-enhancing activity. Benzylphthalimide (P1P-00) exhibited activity which is weaker than that of thalidomide, but introduction of a methyl group at the ortho-position of the benzyl moiety (P1P-10) resulted an increase to a level comparable with that of thalidomide. Phenethylphthalimide (P2P-00) is more potent than thalidomide, and its fluorinated derivative, 2-phenethyl-4, 5, 6, 7-tetrafluoro-1H-isoindole-1, 3-dione (FP2P-00), exhibited potent activity at very low concentrations.—Authors' Abstract

Silva, C. L. New vaccines against tuberculosis. Braz. J. Med. Biolog. Res. **28** (1996) 843–851.

It has proved difficult to vaccinate effectively against tuberculosis with mycobacterial components or even with whole dead mycobacteria; protection was always inferior to that obtained with the live attenuated vaccine known as bacillus Calmette-Guerin (BCG). We have found that this may no longer be the case. Expression of the gene for a single mycobacterial antigen (*Mycobacterium leprae* hsp65) in adult BALB/c mice resulted in substantial cell-mediated protection against challenge with *M. tuberculosis,* but only when it was generated as an endogenous antigen within antigen-presenting cells. CD4 and CD8 T cells cloned from spleens of immunized mice passively transferred protection to nonimmunized mice, and CD8 cells selectively lysed macrophages infected with *M. tuberculosis.* The ability of the clones to protect recipient mice against challenge infection was most strongly associated with specific cytotoxic capacity and secondarily with IFN-gamma production. Three modes of expressing the gene have been tested: a) expression from a retroviral vector (pZIPNeoSV) in implanted J774 tumor cells, b) expression from the same vector via bone marrow cells transfected *in vitro* and used to reconstitute irradiated mice, and c) in a preliminary experiment, from cytomegalovirus (CMV) immediate-early and hydroxymethylglutaryl Co-A reductase promoters injected as plasmid DNA into muscle.—Author's Abstract

Singh, N. B., Srivastava, K., Malaviya, B., Kandpal, H., Srivastava, A. and Gupta, H. P. The 65 kDa protein of *Mycobacterium habana* and its putative role in immunity against experimental tuberculosis. Immunol. Cell Biol. **73** (1995) 372–376.

Mycobacteria including *Mycobacterium tuberculosis* and *M. leprae* possess multiple antigens some of which inhibit other antimycobacterial immune responses. Whole-cell vaccines are not free from these suppressive molecules and may adversely affect the immunogenic response(s). Purified protein components having only immunogenic properties should prove to be superior vaccine(s). *M. habana,* a candidate vaccine for mycobacterial infections, has been dissected for analyzing its antigenic myriad. A 65-kDa protein of this mycobacterium has been isolated and characterized for its protective and cell-mediated immune responses. The protein was isolated in pure form using an isotachophoresis (SDS-PAGE filtration) technique and identified with low molecular weight markers along with monoclonal antibodies (Mab) using the immunoblot technique. Mab IIH9 has identified a 65-kDa protein in *M. habana.* This protein has been found to be immunoprotective in mice against *M. tuberculosis* H37Rv infection. It generates high levels of DTH responses in mice against *M. tuberculosis* and *M. leprae* antigens and inhibits the migration of sensitized cells under the antigenic influence of homologous and heterologous origin. Possibilities of developing this protein as a subunit vaccine are discussed in this report.—Authors' Abstract

Taylor, M. L., Elizondo, N., Mejia Lopez, H., Casasola, J., Martinez Garcia, L. G., Zenteno, E., Salazar, M. A. and Sleman, M. Characterization of an inhibitory seric

factor from tuberculosis anergic patients that acts on non-adherent PPD reactive cells. Immunol. Invest. **24** (1995) 865–879.

Nonadherent cells from PPD+ tuberculosis patients (TBP PPD+) and from healthy individuals treated with whole tuberculosis anergic immune sera or with its protein A-Sepharose IgG fraction, or with sera fraction separated by PPD-Sepharose chromatography, were submitted to immunofluorescence assays. Antihuman IgG or IgM FITC-conjugate were used to reveal the assays, and results were expressed by a fluorescence percentage or fluorescence index. The presence of IgG over the surface of PPD+ nonadherent cells was detected. High fluorescence percentages were observed only in those PPD+ cells treated with whole anergic serum or with its IgG fraction. Positive fluorescence index values were obtained only in those PPD+ cells treated with anergic serum, meanwhile the fluorescence index was always negative when nonbound adherent population are the cell targets for the serum inhibitory factor, which previously has been detected to inhibit antigen response in PPD-reactive cells and point out the specific behavior of this factor, since it was eliminated by PPD-Sepharose chromatography. The IgG nature of the factor was demonstrated by SDS-PAGE and immunoelectrophoresis.—Authors' Abstract

Zaheer, S. A., Beena, K. R., Kari, H. K., Sharma, A. K., Misra, R. S., Mukherjee, A., Mukherjee, R., Kaur, H., Pandey, R. M., Walia, R., Mukhopadhyay, A. and Talwar, C. P. Addition of immunotherapy with *Mycobacterium w* vaccine to multidrug therapy benefits multibacillary leprosy patients. Vaccine **13** (1995) 1102–1110.

Immunotherapy with a vaccine consisting of autoclaved *Mycobacterium w* was given in addition to standard chemotherapy [multidrug therapy (MDT)] to 93 multibacillary (MB) leprosy patients. One-hundred-seven patients with similar types of disease served as controls and received MDT + placebo injections. The study was a double-blind randomized trial. On opening the codes, results obtained were in concordance with those in a single-blind trial which has been extensively reported. Bacteriological clearances were significantly more rapid in vaccinated patients ($p < 0.03$). Thirty-five LL or BL patients with a high bacterial index (BI) of 6 were completely cleared of acid-fast bacilli (AFB) after eight doses of vaccine. Only 8 patients in the control group became bacteriologically negative in the same time period. They all had BIs of <4. Associated with the decrasing BI was accelerated clinicial regression of lesions after vaccination and lepromin conversion rates of 100% for BB, 71% for BL and 70% for LL. A significant number of immunized patients showed histological improvement ($p < 0.004$). Thirty-six showed complete disappearance of dermal granulomas and a picture of nonspecific infiltration. The vaccine did not precipitate pleuritis or deformities; episodes were noted in vaccinated patients as were incidences of type 2 reaction. The overall improvement was reflected by a shorter duration of treatment and faster release of vaccinated patients.—Authors' Abstract

Microbiology

Berthet, F.-X., Rauzier, J., Lim, E. M., Philipp, W., Gicquel, B. and Portnoi, D. Characterization of the *Mycobacterium tuberculosis erp* gene encoding a potential cell surface protein with repetitive structures. Microbiology **141** (1995) 2123–2130.

Using the *phoA* gene fusion methodology adapted to mycobacteria, several *Mycobacterium tuberculosis* DNA fragments encoding exported proteins were recently identified. In this paper, the molecular cloning, genomic positioning, nucleotide sequence determination and transcriptional start site

mapping of a new *M. tuberculosis* gene, identified by this methodology, are reported. This gene was called *erp* (for exported repetitive protein) and has a sequence similar to that of the *M. leprae* 28-kDa antigen *irg* gene. *M. tuberculosis erp* gene contains a putative iron box close to the mapped transcriptional start site. The predicted Erp protein displays a typical N-terminal signal sequence, a hydrophobic domain at the C-terminus and harbors repeated amino acid motifs. These structural features are reminiscent of cell-wall-associated surface proteins from gram-positive bacteria. We found that these repeats are conserved among *M. tuberculosis* isolates, and are absent from the published *M. leprae irg* gene sequence. In addition to being present in *M. leprae, erp* sequences were found in other members of the *M. tuberculosis* complex, but not in other mycobacteria tested. These results suggest that *erp* might encode a cell surface component shared by major pathogenic mycobacteria.—Authors' Abstract

De Beenhouwer, H., Liang, Z., de Rijk, P., van Eekeren, C. and Portaels, F. Detection and identification of mycobacteria by DNA amplification and oligonucleotide-specific capture plate hybridization. J. Clin. Microbiol. **33** (1995) 2994–2998.

We have developed an easy and rapid detection and identification system for the diagnosis of mycobacterial diseases. The system is based on selective amplification by polymerase chain reaction (PCR) of mycobacteria with primers based on the genes coding for 16S rRNA. During PCR, a label (digoxigenin-11-dUTP) is incorporated in the amplicon. After amplification, the amplicon is hybridized in streptavidin-coated microtiter plates prepared with biotinylated species-specific oligonucleotides (oligonucleotide-specific capture plate hybridization [OSCPH]). One oligonucleotide specific for the genus *Mycobacterium* and seven species-specific (*Mycobacterium tuberculosis, M. avium, M. intracellulare, M. scrofulaceum, M. xenopi, M. genavense,* and *M. chelonae*) oligucleotides were designed as capturing probes. After specific hybridization, an enzyme immunoassay reveals the specifically bound complexes and thus permits identification of the mycobacterium. A total of 70 mycobacterial strains were tested. For 69 strains, results concordant with conventional identification were obtained. One *M. chelonae* strain was negative with the *M. chelonae* probe and was later reidentified as *M. fortuitum.* Moreover, for 15 clinicial samples suspected of harboring nontuberculous mycobacteria, OSCPH was able to confirm all culture results and could identify one *M. genavense* infection for which standard culture results were negative. PCR-OSCPH is easily applicable and much faster than culture. It could become a valuable alternative approach for the diagnosis of mycobacterial infections.—Authors' Abstract

Deretic, V., Philipp, W., Dhandayuthapani, S., Mudd, M. H., Curcic, R., Garbe, T., Heym, B., Via, L. E. and Cole, S. T. *Mycobacterium tuberculosis* is a natural mutant with an inactivated oxidative-stress regulatory gene: implications for sensitivity to isoniazid. Mol. Microbiol. **17** (1995) 889–900.

The systems participating in detoxification of reactive oxygen intermediates in *Mycobacterium tuberculosis* are believed to play a dual role in the biology of this highly adapted human pathogen: (i) they may contribute to the survival of this bacterium in the host; and (ii) alterations in the gene encoding catalase/peroxidase have been linked to this organism's resistance to the frontline antituberculosis drug isoniazid. These relationships prompted us to extend investigations of the oxidative-stress-response systems in *M. tuberculosis* by analzing the alkyl hydroperoxide reductase gene *ahpC* and its putative regulatory *oxyR.* Surprisingly, the *oxyR* gene was found to be inactivated by multiple lesions in *M. tuberculosis* H37Rv. These alterations were observed in all *M. tuberculosis* strains tested, and in members of the *M. tuberculosis* complex: *M. bovis* BCG, *M. africanum,* and *M. microti.* The corresponding region carrying these genes in *M. leprae,* an organism not sensitive to isoniazid, has a complete *oxyR* gene divergently transcribed from *ahpC.* An increase in minimal inhibitory concentration for isoniazid was observed upon transformation of *M. tuberculosis* H37Rv with cosmids carrying the *oxyR-ahpC* region of

M. leprae. In keeping with the observed inactivation of *oxyR*, transcriptional activity of the corresponding region in *M. tuberculosis* was an order of magnitude lower than that of the *oxyR* gene from *M. leprae*. While the loss of this putative regulator of oxidative-stress response in *M. tuberculosis* is paradoxical considering the fact that survival in host macrophages is regarded as a critical feature of this pathogen, it offers a partial explanation for the exquisite sensitivity of *M. tuberculosis* to isoniazid.—Authors' Summary

Dhandayuthapani, S., Via, L. E., Thomas, C. A., Horwitz, P. M., Deretic, D. and Deretic, V. Green fluorescent protein as a marker for gene expression and cell biology of mycobacterial interactions with macrophages. Mol. Microbiol. **17** (1995) 901–912.

The green fluorescent protein (GFP) of the jellyfish *Aequorea victoria* offers certain advantages over other bioluminescence systems because no exogenously added substrate or co-factors are necessary, and fluorescence can be elicited by irradiation with blue light without exposing the cells producing GFP to invasive treatments. A mycobacterial shuttle-plasmid vector carrying *gfp* cDNA was constructed and used to generate transcriptional fusions with promoters of interest and to examine their expression in *Mycobacterium smegmatis* and *M. bovis* BCG grown in macrophages or on laboratory media. The promoters studied were: (i) *ahpC* from *Mycoosis* and *M. leprae*, a gene encoding alkyl hydroperoxide reductase which, along with the divergently transcribed regulator *oxyR*, are homologues of corresponding stress-response systems in enteric bacteria and play a role in isoniazid sensitivity; (ii) *mtrA*, an *M. tuberculosis* response regulator belonging to the superfamily of bacterial two-component signal-transcuction systems; (iii) *hsp60*, a previously characterized heat-shock gene from *M. bovis*; and (iv) *tbprc3*, a newly isolated promoter from *M. tuberculosis*. Expression of these promoters in mycobacteria was analyzed using epifluorescence microscopy, laser scanning confocal microscopy, fluorescence spectroscopy, and flow cytometry. These approaches permitted assessment of fluorescence prior to and after macrophage infection, and analyses of promoter expression in individual mycobacteria and its distribution within populations of bacterial cells. Bacteria expressing GFP from a strong promoter could be separated by fluorescence-activated cell sorting from cells harboring the vector used to construct the fusion. In addition, the stable expression of *mtrA-gfp* fusion in *M. bovis* BCG facilitated localization and isolation of phagocytic vesicles containing mycobacteria. The experiments presented here suggest that GFP will be a useful tool for analysis of mycobacterial gene expression and a convenient cell biology marker to study mycobacterial interactions with macrophages.—Authors' Summary

Doukhan, L., Predich, M., Nair, G., Dussurget, O., Mandicmulec, I., Cole, S. T., Smith, D. R. and Smith, I. Genomic organization of the mycobacterial sigma gene cluster. Gene **165** (1995) 67–70.

We have previously described sigma (A) and sigma (B) and their structural genes, mysA and mysB, respectively, in *Mycobacterium smegmatis*. We have now sequenced the corresponding regions in the *M. tuberculosis* and *M. leprae* chromosomes, and have found the two homologous genes. The chromosomal linkage and the deduced amino acid sequences of the two genes show very high similarity in the three species of mycobacteria. We also report the finding of two other open reading frames (ORF) in these clusters. orfX, which has an unknown function, is located between mysA and mysB. The other ORF, located downstream from mysB, encodes a homolog of DtxR, the iron regulatory protein from *Corynebacterium diphtheriae*.—Authors' Abstract

George, K. M., Yuan, Y., Sherman, D. R. and Barry, C. E., III. The biosynthesis of cyclopropanated mycolic acids in *Mycobacterium tuberculosis*; identification and functional analysis of CMAS-2. J. Biol. Chem. **270** (1995) 27292–27298.

The major mycolic acid produced by *Mycobacterium tuberculosis* contains two *cis*-cyclopropanes in the meromycolate chain. The gene whose product cyclopropanates the

proximal double bond was cloned by homology to a putative cyclopropane synthase identified from the *M. leprae* genome sequencing project. This gene, named *cma2*, was sequenced and found to be 52% identical to *cma1* (which cyclopropanates the distal double bond) and 73% identical to the gene from *M. leprae*. Both *cma* genes were found to be restricted in distribution to pathogenic species of mycobacteria. Expression of *cma2* in *M. smegmatis* resulted in the cyclopropanation of the proximal double bone in the α_1 series of mycolic acids. Coexpression of both cyclopropane synthases resulted in cyclopropanation of both centers, producing a molecule structurally similar to the *M. tuberculosis* α-dicyclopropyl mycolates. Differential scanning calorimetry of purified cell walls and mycolic acids demonstrated that cyclopropanation of the proximal position raised the observed transition temperature by 3°C. These results suggested that cyclopropanation contributes to the structural integrity of the cell wall complex.—Authors' Abstract

Guillemin, I., Cambau, E. and Jarlier, V. Sequences of conserved region in the A subunit of DNA gyrase from nine species of the genus *Mycobacterium*: phylogenetic analysis and implication for intrinsic susceptibility to quinolones. Antimicrob. Agents Chemother. **39** (1995) 2145–2149.

The sequences of a conserved region in the A subunit of DNA gyrase corresponding to the quinolone resistance-determining region were determined for nine mycobacterial species and were compared. Although the nucleotide sequences were highly conserved, they clearly differentiated one species from another. The results of the phylogenetic analysis based on the sequences of the quinolone resistance-determining regions were compared with those provided by the 16s rRNA sequences. Deduced amino acid sequences were identical within the nine species except for amino acid 83, which was frequently involved in acquired resistance to quinolones in many genera, including mycobacteria. The presence at position 83 of an alanine for seven mycobacterial species (*M. tuberculosis, M. bovis* BCG, *M. leprae, M. avium, M. kansasii, M. chelonae,* and *M. smegmatis*) and of a serine for the two remaining mycobacterial species (*M. tuberculosis* and *M. aurum*) correlated well with the MICs of ofloxacin for both groups of species, suggesting the role of this residue in intrinsic susceptibility to quinolones in mycobacteria.—Authors' Abstract

Harris, D. P., Vordermeier, H. M., Singh, M., Moreno, C., Jurcevic, S. and Ivanyi, J. Cross-recognition by T cells of an epitope shared by two unrelated mycobacterial antigens. Eur. J. Immunol. **25** (1995) 3173–3179.

The molecular mimicry represented by cross-recognition of determinants shared by unrelated antigens by antibodies or T cells is of broad immunological interest. In this study, we analyzed the cross-recognition by CD4+ T cells of a peptide epitope shared by two mycobacterial proteins of diverse sequence, represented by the 19-kDa antigen of *Mycobacterium tuberculosis* and the 28-kDa antigen of *M. leprae*. This epitope was immunodominant with respect to the 19-kDa antigen, but cryptic in relation to the 28-kDa antigen. The crossreactive epitope cores were identified by Pepscan window analysis and found to be eight residues long in both antigens (residues 69-76 and 127-134). Alignment of these octameric sequences revealed two identical and five conservatively related amino acids. Within the editope core, two residues [(73)Asn and (76)Ile] were identified as critical for recognition on the basis of inhibition of the crossreactive T-cell proliferative response using singly substituted analog peptides. These results suggest that T-cell crossreactive epitopes can exist in proteins with apparently not more than random levels of sequence homology. Their potential for unsuspected cross-sensitization may play a role in the maintenance of T-cell memory, in the pathogenesis of autoimmune diseases and possibly in a wide range of host immune responses to infectious pathogens.—Authors' Abstract

Huberts, P. and Mizrahi, V. Cloning and sequence analysis of the gene encoding the DNA polymerase I from *Mycobacterium tuberculosis*. Gene **164** (1995) 133–136.

The polA gene (encoding DNA polymerase I) from *Mycobacterium tuberculosis* was cloned using an internal gene segment probe generated by PCR amplification on genomic DNA [Mizrahi, *et al.*, Gene 136 (1993) 287–290]. The gene encodes a polypeptide 904 amino acids (aa) in length that shares 89% identity with a 911-aa homologue from *M. leprae*. The polypeptide has all of the primary structural elements necessary for DNA polymerase and 5′-3′ exonuclease activity, but lacks the motifs required for an associated 3′-5′ exonuclease (proofreading) activity.—Authors' Abstract

Kremer, L., Baulard, A., Estaquier, J., Poulain-Godefroy, O. and Locht, C. Green fluorescent protein as a new expression marker in mycobacteria. Mol. Microbiol. **17** (1995) 913–922.

This study describes the use and the advantages of the green fluorescent protein (GFP) as a reporter molecule for mycobacteria. The *gfp* gene from *Aequorea victoria* was placed under the control of the hsp60 promoter in the shuttle vector pGFM-11. The *gfp* expression in the recombinant *Mycobacterium smegmatis* and BCG was readily detected on agar plates by the development of an intense green fluorescence upon irradiation with long-wave u.v. light. In mycobacteria containing a pGFM-11 derivative that lacks the hsp60 promoter, no fluorescence was observed. However, this plasmid was successfully used as a promoter-probe vector to identify BCG promoters. The fluorescence emission of GFP in mycobacteria harboring pGFM-11 and grown in liquid media could be quantified by spectrofluorimetry. This allowed for easy assessment of drug susceptibility. Since GFP does not require the addition of substrates or co-factors, the green fluorescent bacilli could be directly observed within infected macrophages using fluorescence and laser confocal microscopy, or in tissue sections of infected mice. Finally, infected cells or free-living recombinant mycobacteria could also be analyzed by flow cytometry. The GFP thus appears to be a convenient reporter for mycobacteria, allowing tracing of recombinant mycobacteria, isolation of promoters with interesting properties, *in vivo* drug testing and the development of new diagnostic tools.—Authors' Summary

Laqueyrerie, A., Militzer, P., Romain, F., Eiglmeier, K., Cole, S. and Marchal, G. Cloning, sequencing, and expression of the *apa* gene coding for the *Mycobacterium tuberculosis* 45/47-kilodalton secreted antigen complex. Infect. Immun. **63** (1995) 4003–4010.

Effective protection against a virulent challenge with *Mycobacterium tuberculosis* is induced mainly by previous immunization with living attenuated mycobacteria, and it has been hypothesized that secreted proteins serve as major targets in the specific immune response. To identify and purify molecules present in culture medium filtrate which are dominant antigens during effective vaccination, a two-step selection procedure was used to select antigens able to interact with T lymphocytes and/or antibodies induced by immunization with living bacteria and to counterselect antigens interacting with the immune effectors induced by immunization with dead bacteria. A *M. bovis* BCG 45/47-kDa antigen complex, present in BCG culture filtrate, has been previously identified and isolated (F. Remain, A. Laqueyrerie, P. Militzer, P. Pescher, P. Chavarot, M. Lagranderie, G. Auregan, M. Gheorghiu, and G. Marchal, Infect. Immun. 61:742–740, 1993). Since the cognate antibodies recognize the very same antigens present in *M. tuberculosis* culture medium filtrates, a project was undertaken to clone, express, and sequence the corresponding gene of *M. tuberculosis*. An *M. tuberculosis* shuttle cosmid library was transferred in *M. smegmatis* and screened with a competitive enzyme-linked immunosorbent assay to detect the clones expressing the proteins. A clone containing a 40-kb DNA insert was selected, and by means of subcloning in *Escherichia coli*, a 2-kb fragment that coded for the molecules was identified. An open reading frame in the 2061-nucleotide sequence codes for a secreted protein with a consensus signal peptide of 39 amino acids and a predicted molecular mass of 28, 779 Da. The gene was referred to as *apa* because of the high percentages of proline (21.7%) and alanine (19%) in the purified protein. Southern hybridization analysis of digested total genomic DNA from *M. tuberculosis* (reference strains H37Rv and H37Ra) indicated that the *apa* gene was present as a single copy

on the genome. The N-terminal identity or homology of the *M. tuberculosis* and *M. bovis* BCG purified molecules and their similar global and deduced amino acid compositions demonstrated the perfect correspondence between the molecular and chemical analyses. The presence of a high percentage of proline (21.7%) was confirmed and explained the apparent higher molecular mass (45/47 kDa) determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis resulting from the increased rigidity of molecules due to proline residues. Extensive homology with the 43L *M. leprae* antigen (B. Wieles, M. van Agterveld, A. Janson, J. Clark-Curtiss, T. Rinke de Wit, M. Harboe, and J. Thole, Infect. Immun. 62:252–258, 1994) and the capacity to induce a potent T- and-dependent response prompted us to analyze the role of the protein(s) for the bacteria and its effect on the immune response during *M. tuberculosis* infection.—Authors' Abstract

Li, T., *et al.* [Optimization of conditions for PCR of *M. leprae* and its relevant factors.] China Lepr. J. **11** (1995) 124–126. (in Chinese)

After selected simple melt-freeze method of sample preparations for polymerase chain reaction (PCR) and optimized its cyclic parameters, sensibility of the PCR has been significantly increased, from 20 bacilli/μl to 0.2 bacillary/μl, which were proved in the tests using 16 samples of nude mice foot pads and 26 ones of leprosy patients. The perspective of applying the PCR in detection and identification of *M. leprae* is discussed.—Authors' English Abstract

Musser, J. M. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin. Microbiol. Rev. **8** (1995) 496.

The primary theme emerging from molecular genetic work conducted with *Mycobacterium tuberculosis* and several other mycobacterial species is that resistance is commonly associated with simple nucleotide alterations in target chromosomal genes rather than with acquisition of new genetic elements encoding antibiotic-altering enzymes. Mutations in a 81-bp region of the gene (rpoB) encoding the beta subunit of RNA polymerase account for rifampin resistance in 96% of *M. tuberculosis* and many *M. leprae* isolates. Streptomycin resistance in about one-half of *M. tuberculosis* isolates is associated with missense mutations in the rpsL gene coding for ribosomal protein S12 or nucleotide substitutions in the 16S rRNA gene (rrs). Mutations in the katG gene resulting in catalase-peroxidase amino acid alterations and nucleotide substitutions in the presumed regulatory region of the inhA locus are repeatedly associated with isoniazid-resistant *M. tuberculosis* isolates. A majority of fluoroquinolone-resistant *M. tuberculosis* isolates have amino acid substitutions in a region of the DNA gyrase A subunit homologous to a conserved fluoroquinolone resistance-determining region. Multidrug-resistant isolates of *M. tuberculosis* arise as a consequence of sequential accumulation of mutations conferring resistance to single therapeutic agents. Molecular strategies show considerable promise for rapid detection of mutations associated with antimicrobial resistance. These approaches are now amenable to utilization in an appropriately equipped clinical microbiology laboratory.—Author's 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 macrolide resistance in *M. avium* and also identifies a possible molecular mechanism of resistance at the level of the ribosome.—Authors' Abstract

Philipp, W. J. and Cole, S. T. Local comparison of the genomes of *Mycobacterium tuberculosis* and *Mycobacterium leprae* using the polymerase chain reaction. FEMS Microbiol. Lett. **132** (1995) 263–269.

To facilitate comparison of the genome maps of *Mycobacterium tuberculosis* and *M. leprae*, sequence data from the *M. leprae* sequencing project were used to design primers suitable for the amplification of short segments from conserved genes by the polymerase chain reaction. In most cases, both organisms yielded products of identical size that were then used as probes for a comparative genomic walk. The hybridization data often, but not always, revealed a similar organization of various regions of both genomes. This approach should be useful for systematic comparisons of (myco)bacterial genomes.—Authors' Abstract

Sharma, R. K., Katoch, K., Sharma, V. D., Shivannavar, C. T., Natarajan, M. and Katoch, V. M. Isolation and characterization of cultivable mycobacteria from leprosy skin. Indian J. Lepr. **67** (1995) 321–328.

Attempts were made to isolate cultivable mycobacteria from 129 biopsies/slit-skin scrapings from the skin of leprosy patients (73 multibacillary—BB/BL/LL—and 56 paucibacillary—TT/BT/I) as well as 50 healthy controls. Among the 19 isolates obtained, 17 were from specimens from leprosy cases whereas two were from healthy controls: 14 of the 17 isolates were from multibacillary cases and three were from paucibacillary patients. The mycobacteria isolated were: *Myhcobacterium scrofulaceum* (4 = all LL cases); *M. avium* (3 = 2 from LL cases and 1 from healthy control); *M. avium-intracellulare* complex (1 LL); *M. gordonae* (2 = 1 from BT and BB each); *M. flavescens* (1 BL); *M. smegmatis* (2 = both LL); *M. phlei* (4 = 1 LL, 1 BL, 1 BT and 1 healthy control); *M. fortuitum* (1 BL); and *M. chelonei* (1 BT relapse). The results of this study suggest a preferential colonization of skin of lepromatous leprosy cases by *M. scrofulaceum* and *M. avium*. As such isolates have been reported by the investigators from other parts of the world, independent confirmation of such trends in Indian patients is significant and the causal relationship (if any) between such colonization and development of lepromatous disease merits further investigation.—Authors' Abstract

Sharma, R. K., Katoch, K., Sharma, V. D., Shivannavar, C. T., Natarajan, M. and Katoch, V. M. Studies on microbial aerobic flora of skin in leprosy patients. Indian J. Lepr. **67** (1995) 309–319.

This study reports the isolation and identification of aerobic organisms from biopsies/slit-skin smears/scrapings from 129 leprosy patients and 50 healthy controls. These include 56 paucibacillary (PB) and 73 multibacillary (MB) cases. Thirty-six isolates from the specimens from 21 patients and 15 healthy controls were grown. The non-mycobacterial isolates from clinically PB leprosy (TT/BT/I) patients were: (a) gram-positive cocci: *Staphylococcus aureus*(1), *Staphylococcus albus*(1); (b) gram-positive bacilli: *Bacillus subtilis*(1), *Corynebacterium xerosis*(1) and (c) gram-negative bacilli: *Escherichia coli*(1), *Proteus mirabilis*(2), *Klebsiella pneumoniae*(1) and *Pseudomonas aeruginosa*(1). The isolates from clinicially MB leprosy (BB/BL/LL) patients were: (a) gram-positive cocci: *Micrococci*(1), *Staphylococcus aureus*(1) and *Staphylococcus albus*(1); (b) gram-positive bacilli: *Corynebacterium xerosis*(1); *Cory-*

nebacterium hofmanni(1) and *Bacillus cereus*(1) and (c) gram-negative bacilli: *Escherichia coli*(2), *Klebsiella pneumoniae*(1) and *Proteus mirabilis*(2). The specimens from healthy controls yeilded similar organisms. These were: (a) gram-positive cocci: *Staphylococcus albus*(2), *Staphylococcus aureus*(2) and *Micrococci*(2); (b) gram-positive bacilli: *Corynebacterium xerosis*(1) and (c) *Bacillus subtilis*(2), *Corynebacterium hofmanni*(1) and *Bacillus cereus*(1) and (c) gram-negative bacilli: *Escherichia coli*(3), *Proteus vulgaris*(1) and *Proteus mirabilis*(1). While these results show no significant differences in the species types of non-mycobacterial aerobic organisms isolated from healthy skin and PB/MB types of leprosy, these isolates need to be characterized by immunological/molecular methods to find out subtypes if any.—Authors' Abstract

Wieles, B., Van Noort, J., Drijfhout, J. W., Offringa, R., Holmgren, A. and Ottenhoff, T. H. M. Purification and functional analysis of the *Mycobacterium leprae* thioredoxin/thioredoxin reductase hybrid protein. J. Biol. Chem. **270** (1995) 25604–25606.

In *Mycobacterium leprae*, thioredoxin and thioredoxin reductase are expressed from a single gene. This results in the expression of a hybrid protein with subunits attached to each other by a hydrophilic peptide linker. In all other organisms studied so far, thioredoxin (Trx) and thioredoxin reductase (TR) are expressed as two separate proteins. This raises the question of whether the hybrid protein is enzymatically active and, if so, whether TR reduces its own Trx partner or, alternatively, a heterologous Trx subunit. To address this question, the hybrid TR/Trx protein of *M. leprae* as well as the individual parts of the hybrid gene coding for either TR or Trx were overexpressed in *Escherichia coli* and purified. The purified proteins were tested for their ability to catalyze NADPH-dependent insulin disulfide reduction. Here we show that the *M. leprae* hybrid protein is indeed enzymatically active. Compared with the enzymatic activity of the separately expressed Trx and TR proteins, the hybrid protein is shown to be more efficient, particularly at low equimolar concentrations. This suggests that the hybrid protein of *M. leprae* is active by itself and that its activity involves intramolecular interactions between the TR and Trx domains. The activity of the hybrid protein increases when exogenous TR or Trx is added, indicating an additional role for intermolecular interactions.—Authors' Abstract

Experimental Infections

Agnihotri, N., Ganguly, N. K., Kaur, S., Khullar, M., Sharma, S. C. and Chugh, K. S. Role of reactive oxygen species in renal damage in experimental leprosy. Lepr. Rev. **66** (1995) 201–209.

Renal involvement is known to occur in leprosy. In the present study the possible role of reactive oxygen species (ROS) in causation of renal damage in mice infected with *Mycobacterium leprae* has been investigated. At least six animals from each group (control and infected) were killed at 0 day, 3, 6 and 9 months postinfection. The results showed a significant increase in the chemiluminescence (CL) response of peritoneal macrophages which was maximum between 3 and 6 months. No significant increase was observed in CL response of blood neutrophils. A significant increase in lipid peroxidation was observed at 3 and 6 months as evident by an increase in malondialdehyde levels. The increased ROS production might be the cause of lipid peroxidation. The renal damage is also evident by decrease in the activity of renal brush border membrane enzymes, namely, alkaline phosphatase, leucine aminopeptidase and r-glutamyl transpeptidase. Thus ROS might play a role during early stages of *M. leprae* infection but in the later stages other immunological mechanisms may overpower the effect of ROS.—Authors' Summary

Epidemiology and Prevention

Dayal, R. and Bharadwaj, V. P. Prevention and early detection of leprosy in children. J. Trop. Pediatr. **41** (1995) 132–138.

The authors report a 5-year follow-up study of 455 healthy children who were close contacts of leprosy patients in Uttar Pradesh, India. On the basis of results of the fluorescent leprosy antibody absorption technique (FLA-ABS) and lepromin tests, these 455 contacts were classified into four groups: Group I, comprising children who were FLA-ABS positive and lepromin positive; Group II, who were FLA-ABS positive and lepromin negative; Group III, who were FLA-ABS negative and lepromin positive; and Group IV, who were FLA-ABS negative and lepromin negative. During the follow-up period of 5 years, only 2 out of 155 children in Group I developed the disease showing that their good cell-mediated immunity had been able to contain the disease. Out of 166 contacts in Group II, 18 developed the disease mainly of the tuberculoid type. Most of these children were contacts of multibacillary patients. None of the children in Groups III and IV developed the disease. These findings were statistically significant ($p < 0.01$). Out of the 166 children in Group II (the "at risk" group), 70 were treated as controls while 96 were put on prophylaxis with dapsone which was continued for 3 years after the contact with the source patient had ceased, or for 3 years after the source patient became noninfective. The incidence of disease was significantly lower among children who received chemoprophylaxis ($p < 0.05$). Among controls the incidence rate of the disease was higher in contacts below 5 years of age, males, and contacts of bacteriologically positive patients ($p < 0.05$). These contacts were labelled as the "high risk" group. The efficacy rate of dapsone prophylaxis was significantly better in: contacts below 5 years of age as compared to older contacts; males as compared to females; contacts of bacteriologically positive patients; and contacts whose source patients were under treatment. This study demonstrates the value of the FLA-ABS and lepromin tests in detecting subclinical infection and for identifying the "at risk" contacts of leprosy patients. It clearly establishes the chemoprophylactic value of dapsone for the "at risk" contacts, particularly for those in the "high risk" category. In pursuance of the Indian government's policies under the National Leprosy Eradication Programme, this study suggests the need to carry out surveillance surveys in the endemic population to identify, follow, and offer chemoprophylaxis to those at risk.—Authors' Abstract

Hatta, M., Van Beers, S. M., Madjid, B., Djumadi, A., de Wit, M. Y. L. and Klatser, P. R. Distribution and persistence of *Mycobacterium leprae* nasal carriage among a population in which leprosy is endemic in Indonesia. Trans. R. Soc. Trop. Med. Hyg. **89** (1995) 381–385.

In order to understand better the relationship among *Mycobacterium leprae,* its transmission and the human host or the chain of infection which may lead to the development of leprosy, we performed a population survey in which nasal carriage of *M. leprae* was determined by a specific polymerase chain reaction (PCR) 2 years after an earlier survey in the same population: 1923 persons were registered, 1171 were clinically examined for signs of leprosy, and 418 were tested by PCR. The detection rate of leprosy in the study area had not changed significantly during the 2-years observation period since the introduction of multidrug therapy, i.e., 6/1000 compared to 7.7/1000 2 years before. Of 6 newly detected cases, 5 were diagnosed as having paucibacillary leprosy. The presence of *M. leprae* could be demonstrated by PCR in 2.9% (12/418) of the persons. PCR positivity was not persistent over the 2 years. All the PCR-positive persons identified in the first survey were negative in the second, indicating that *M. leprae* nasal carriage is transient. As in the previous survey, we found evidence for widespread *M. leprae* nasal carriage as determined by PCR among the general population in an area in which leprosy is endemic. In addition, our data indicated that PCR positivity can occur in certain clusters in the community. This

clustering seems to be time-dependent, not necessarily related to the presence of patients.—Authors' Abstract

Ma, W., *et al.* [Possibility of basically eradicating leprosy by the end of 1995 in Guangzhou.] China Lepr. J. **11** (1995) 74–75. (in Chinese)

In the last 35 years there accumulatively were 7399 registered cases of leprosy in the city of Guangzhou. By the end of 1993 there remained 58 active cases and the prevalence was 0.015 per 1000, decreasing by 98.7% as compared with that in the year with the highest one. In the same period of time the incidence also declined by 99.8%; there were no new childhood cases and those with the disease duration of less than 2 years among the new cases were increasing. It is estimated that the aim at basic eradication of leprosy, i.e., the prevalence and incidence are less than 0.01 per 1000 and 0.5 per 100,000, respectively, will be reached in the jurisdictional area of Guangzhou by the end of 1995.—Authors' English Abstract

Pan, S. [Endemicity of leprosy both 10 years before and after MDT.] China Lepr. J. **11** (1995) 79–80. (in Chinese)

Out of the population of 1.5 million, 2785 cases of leprosy have been found from the beginning of the 1960s to the end of 1993 in Xinghua City, Jiangsu, and the highest prevalence and incidence were 1.75 per 1000 and 19.62 per 100,000, respectively. Since 1983 the WHO-MDT regimen has been adopted. For 10 years before MDT and in 10 years afterward 283 and 65 new patients were found, respectively. As compared with those before MDT, for the 10 years after MDT among new patients the proportion of MB cases and grade 2 and 3 disability increased, but the disease duration shortened and childhood rate and family attack decreased.—Author's English Abstract

Rao, P. S., Subramanian, M., Subramanian, G. and Parkash, I. Prospects for elimination of leprosy in India by 2000 AD. Indian J. Lepr. **67** (1995) 285–292.

Data regarding the trends of new-case detection rates of leprosy for India as a whole, for the state of Andhra Pradesh, and for Srikakulam district in Andhra Pradesh were generated and projected up to 2000 AD. The prevalence rate by 2000 AD was worked out based on these new-case detection rates. The projects show that at the current slowly declining trend of new-case detection, with 20% MB cases among the newly detected cases and the current mean duration of treatment, the elimination goal of leprosy by 2000 AD could possibly be achieved at Srikakulam district level only, where the MDT project has been under implementation for over 10 years, but not at the state or country levels. The achievement of the elimination goal should be possible in other geographic units also if the duration of disease could be shortened to 1 month or less, for both paucibacillary and multibacillary types of leprosy.—Authors' Abstract

Shen, L., *et al.* [A strengthened health education on leprosy.] China Lepr. J. **11** (1995) 136–138. (in Chinese)

A strengthened health education on leprosy for 1 week to 1 month under the leadership of the local health authorities has been carried out in three cities, Tongxiang, Cixi and Shaoxing, Zhejiang Province, in 1994, aimed at community leaders, medical workers in town and village health centers, inhabitants and children in primary and middle schools, respectively, by using all available means. The evaluations of this education with inquiry and questionnaire showed that knowledge on leprosy has been widely popularized in the population.—Authors' English Abstract

Yao, J. [A study of influence of socioeconomic factors on endemicity of leprosy with a method of obscure mathematics.] China Lepr. J. **11** (1995) 68–72. (in Chinese)

Influence of various socioeconomic factors on endemicity of leprosy was retrospectively investigated in 157 persons with active leprosy and cures in Human Province by using a method of obscure mathematics. The author noticed that the majority of them were and are living under conditions lower than local moderate living standard with less income, low educational level, no paying attention to hygiene, moist resident environment and engaging in agriculture, and

pointed out that poverty and ignorance must be eliminated for eradicating leprosy.—Author's English Abstract

Zhen, Y., *et al.* [Effect of MDT for eight years.] China Lepr. J. **11** (1995) 127–128. (in Chinese)

In Anhui Province since the introduction of WHO's MDT in 1986, up to the year 1993, 1520 patients with leprosy have completed the course of treatment, including MB 690 and PB 830. There were 1197 active cases of leprosy, including MB 574 and PB 623, in 1986 and there remain 261 cases, including MB 182 and PB 79, so the prevalence decreased from 0.026‰ in 1985 to 0.0039‰ in 1993, and the endemic area is obviously reduced. All 81 counties and cities had achieved the goal of basic eradication of leprosy in 1993.—Authors' English Abstract

Rehabilitation

Kopparty, S. N. M. Problems, acceptance and social inequality: a study of the deformed leprosy patients and their families. Lepr. Rev. **66** (1995) 239–249.

Although the impact of social inequality on health conditions is widely known, its impact on the chronic and stigmatized disease leprosy has received little attention. Deformity sometimes leads to disabilities and to handicaps causing problems to the patient and his family. In this paper an attempt has been made to understand the impact of social inequality, prevalent in the form of the caste system in India, on the deformed leprosy patients and on their families. This impact was examined in terms of the problems faced by the patients. A sample of 150 deformed patients and their families, drawn from two districts in Tamil Nadu, was selected for the study.

About 57% of the deformed patients experienced their deformity as a handicap which caused social and economic problems while the rest did not. Of the three caste groups, the Lower Caste group experienced more severe economic problems while the Upper Caste group faced more social problems. The extent of acceptance of deformed patients in their family varied significantly among those facing and not facing problems due to their deformity. The deformed patients without any handicap were accepted in a large majority of their families (82%) regardless of their caste status. In contrast the deformed but handicapped patients were accepted differentially among the three caste groups with the Upper group accepting them in most of their families (80%) while in the Lower group much fewer of families (54%) did. All the families of the deformed but not handicapped patients desired to keep their patients until their death, irrespective of their caste status. On the contrary, while all the families in the Upper Caste group expressed their willingness to keep their handicapped patients in the family until their death, 10% in the Middle and 22% in the Lower Caste groups did not want to do so. This suggests the gradual marginalization, rejection and dehabilitation of the affected. Thus, one's caste status can be a broad indicator of the nature and extent of handicaps and acceptance in the family. This factor needs to be appropriately taken care of for rehabilitation and disbility management in leprosy control programs.—Author's Summary

Qian, J. [Skin flap with retrograde pedicle of medial plantar artery and its clinical application.] China Lepr. J. **11** (1995) 116–117. (in Chinese)

Skin flap with retrograde arterial pedicle taken from the medial part of the sole has been used to repair plantar ulcers in two cases of leprosy. Follow up for 6 months to 2 years showed no relapse of the ulcers.—Author's English Abstract

Qian, J., *et al.* [Application of a modified skin flap to the leg amputation in leprosy.]

China Lepr. J. **11** (1995) 63–64. (in Chinese)

For amputation of the leg, a modified skin flap was designed. The longer one out of two flaps bears fascia flap longer than it and can be inserted under the short skin flap on suturing so as to increase the thickness of overlying soft tissue on the stump of the fibia for prevention of ulceration originating from oppression and friction.—Authors' English Abstract

Shanghai Zunyi Hospital. [Anatomic study and clinical use of skin flap of dorsum pedis.] China Lepr. J. **11** (1995) 114–116. (in Chinese)

A skin flap with the dorsal artery of the foot as a pedicle has longer pedicle with thick arterial vessel, better elasticity of the skin and sensory nerve supply so as to constitute a good donor for repair of plantar ulcers in leprosy. Since 1974, the authors have repaired ulcers for 30 leprosy patients with the skin flap with follow up of 36 to 120 months; only one case was seen with relapse of the ulcer. Because this skin flap is very thin, the flap must be taken close to the periosteum otherwise the artery might become separated from the skin.—Authors' English Abstract

Tang, X. [Follow up of the cases of lagophthalmos corrected with nylon filament method.] China Lepr. J. **11** (1995) 73–74. (in Chinese)

A nylon filament method for correcting lagophthalmos has been more extensively used for leprosy pateints for the last 10 years and more in china. From May 1994 to June 1995, the author examined 92 eyes of 52 cases who had received the operation 2.5 to 25 years ago in Guangdong. No eye of them can entirely close its palpebral fissue and the fissures remain over 6 mm at closing the eye in 81 eyes of 46 cases. On the basis of the records of case history the fissures became wider than those before the operation in 32 eyes of 19 persons, and it was also noted that 2 weeks after the operation the nylon filament began to be exposed or loosening and even to be infected although the size of palpebral fissures has, to some extent, lessened in a shorter duration after the operation.—Author's English Abstract

Yang, P. [Surgical treatment of ankle joint luxation and fixed talipes equinovarus with ulcer on foot outside in a case of leprosy.] China Lepr. J. **11** (1995) 78–79. (in Chinese)

Surgical treatment has been given to a leprosy patient with luxation of the ankle joint, fixed talipes equinovarus and a large ulcer on the outside of the foot. At first, the ulcer had been healed and then arthrodesis of the ankle joint and three joints of the foot was completed. So, he has gained the ability to live by his hands.—Author's English Abstract

Yu, Z., *et al.* [Chronic osteomyelitis complicated to plantar ulcer in 102 cases of leprosy.] China Lepr. J. **11** (1995) 77–78. (in Chinese)

Since 1983, 216 feet with plantar ulcers in 204 cases of leprosy have been observed; 112 feet of 102 cases among them suffered from chronic osteomyelitis, including 34 paucibacillary and 68 multibacillary leprosy cases with a mean age of 41 years. The osteomyelitis increased along with the ulcer duration. Among them, 82 cases died: 5 from pyosepticemia, 5 from neoplasia and metastasis, and 1 from hematorrhea. Seventy-six legs in 70 cases have been amputated for pyosepticemia, hematorrhea, necrosis of a large area, serious deformity, or neoplasia. The authors emphasize the importance of preventing the ulcer and self-care of the feet.—Authors' English Abstract

Zhen, Z. [Effects of self-care in inpatients with leprosy for three years.] China Lepr. J. **11** (1995) 121–123. (in Chinese)

Since October of 1990, 69 persons who have and had leprosy have been taught self-care for their disabled hands, feet and eyes by explaining, demonstrating and directing the operations for 5 weeks. The majority of them have cultivated habits of self-care. After 3 years, follow up showed that inflammation of the eye resolved in 19 of 27 cases and fissures of the hands in 32 out of 42,

and plantar ulcer in 25 out of 30 have healed.—Author's English Abstract

Zhou, L., *et al.* [Disability among 27,782 leprosy patients.] China Lepr. J. **11** (1995) 118–120. (in Chinese)

A survey of disability in 27,782 active and arrested leprosy patients out of 28,006 living now shows that 14,577 cases (52.46%) have deformities and disabilities, of which 88.7% are grades 2 and 3. What was most often seen is madarosis, claw hand and ape palm. The disabilities have been increasing with age and disease duration, and are more often found in those who are irregularly treated, in peasants, and in the borderline tuberculoid form of the disease.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Aarestrup, F. M., Goncalves da Costa, S. C. and Sarno, E. N. The effect of thalidomide on BCG-induced granulomas in mice. Braz. J. Med. Biol. Res. **28** (1995) 1069–1076.

Granuloma proliferation is the result of a series of complex biological events in which a variety of cell types and cytokines are involved. Tumor necrosis factor alpha (TNF-α) plays a central role. In the present study, we investigated the effect of thalidomide (α-N-pthalimidoglutarimide), a selective inhibitor of TNF-α synthesis, on granuloma formation during BCG infection in Oncins France 1 (OF-1) mice. Subcutaneous injections of 30 mg/kg body weight of thalidomide daily for 14, 21 or 28 days into the mice resulted in the reduction of the size and total number of liver granulomas. The most striking effect of thalidomide was observed after 28 days, when the total number of liver granulomas was reduced by as much as 40% ($p < 0.05$). Serum TNF-α levels of thalidomide-treated mice were significantly lower (85%) than control mice on day 14 and remained lower (55%) on days 21 and 28. Positive immunohistochemical staining specific for TNF-α was demonstrable only in well-developed granulomas in which central mononuclear cells presented extensive differentiation into epithelioid cells. Daily administration of thalidomide for 21 to 28 days to the BCG-infected mice inhibited local TNF-α expression in well-developed granulomas. The mechanisms by which thalidomide modulates the granuloma proliferation are discussed.—Authors' Abstract

Agrewala, J. N. and Mishra, G. C. A 38-kDa antigen of *Mycobacterium tuberculosis* predominantly induces the secretion of interleukin-2, interferon-gamma and IgG2a antibodies. Microbiol. Immunol. **39** (1995) 801–808.

In the present study, mice of three different haplotypes (H-2d, H-2k and H-2b) were sensitized subcutaneously with heat-killed H37Ra or 38-kDa antigen of *Mycobacterium tuberculosis*. Lymphocytes obtained from immunized animals were challenged *in vitro* with 38-kDa antigen in both cases. The dominant pattern of Th1-like lymphokines (IL-2 and IFN-gamma) and preferential production of 38-kDa specific IgG2a-type antibody were observed. It was noted that 38-kDa antigen was recognized permissively by all three strains of mice used in the present study. It was interesting to note that C3H/HeJ mice, which express BCG-resistant alleles, showed a higher level of proliferative as well as cytokine response as compared to BALB/c and C57BL/6 mice, which bear BCG-susceptible alleles. These results suggest that not only in recall responses but also during the induction as well as expression phase of the immune response mediated by 38-kDa antigen of *M. tuberculosis* the Th1-like immune response predominates.—Authors' Abstract

Armoa, G. R. G., Rouse, D. A., Nair, J., Mackall, J. C. and Morris, S. L. A highly immunogenic putative *Mycobacterium kansasii* lipoprotein. Microbiology (U.K.) **141** (1995) 2705–2712.

The resurgence of tuberculosis, the emergence of multiple-drug-resistant tuberculosis, and the increasing prevalence of mycobacterial disease in AIDS patients have increased the importance of defining new mycobacterial antigens that can be utilized in the development of improved diagnostic reagents and more effective vaccines. In this report, a highly immunogenic *Mycobacterium kansasii* protein (MK35) and the gene encoding this antigen were characterized. MK35 gene probes reacted with genomic DNA from *M. avium*, *M. bovis* BCG, *M. intracellulare* and *M. tuberculosis* but not with DNA isolated from nine other mycobacterial species. Nucleotide sequence analysis showed that the MK35 gene encodes a 26-kDa protein which contains a consensus bacterial lipoprotein processing sequence. In addition, detergent-phase separation studies strongly suggested that MK35 is a lipoprotein. Skin-test assays demonstrated that MK35 elicited a strong response in guinea pigs sensitized with *M. kansasii* but did not read in *M. tuberculosis*-sensitized guinea pigs. These results further suggest that mycobacterial lipoproteins are immunogenic antigens that should be considered in the development of new mycobacterial vaccines and diagnostic reagents.—Authors' Abstract

Arriaza, B. T., Salo, W., Aufderheide, A. C. and Holcomb, T. A. Pre-Columbian tuberculosis in northern Chile: molecular and skeletal evidence. Am. J. Phys. Anthropol. **98** (1995) 37–45.

Analysis of 483 skeletons from Arica (Chile) and review of mummy dissection records demonstrates an overall 1% prevalence rate for tuberculosis between 2000 B.C. and 1500 A.D. Tuberculosis cases cluster in the period 500–1000 A.D. which correlates with fully agropastoral societies. Considering only these agropastoral societies, about 2% of their members show tuberculosis lesions. A segment of DNA unique to *Mycobacterium tuberculosis* was identified in an extract from the vertebral lesion of a 12-year-old girl with Pott's disease from about 1000 A.D., establishing the pre-Columbian presence of tuberculosis with the most specific evidence currently available.—Authors' Abstract

Atkinson, B. A., Bocanegra, R. and Graybill, J. R. Treatment of *Mycobacterium haemophilum* infection in a murine model with clarithromycin, rifabutin, and ciprofloxacin. Antimicrob. Agents Chemother. **39** (1995) 2316–2319.

An animal model of disseminated *Mycobacterium haemophilum* infection was utilized to compare treatment with azithromycin, ciprofloxacin, rifabutin, and the combination of clarithromycin with rifabutin. Following subcutaneous challenge with *M. haemophilum*, local and disseminated infection occurred only in immunosuppressed mice. For disseminated infection, ciprofloxacin was relatively ineffective therapy. Clarithromycin and rifabutin alone significantly reduced the tissue burden in the spleen after 4 weeks of therapy. Combination therapy with rifabutin and clarithromycin was superior to 4 weeks of treatment with the individual agents. When immunosuppressed mice were treated for 20 weeks with the combination of rifabutin and clarithromycin, the tissue burden remained reduced in the spleen at 1 month following the completion of therapy. Combined rifabutin and clarithromycin provide effective treatment for *M. haemophilum* in this model.—Authors' Abstract

Banerjee, P., Chakrabarty, A. N. and Dastidar, S. G. Propagation of *Mycobacterium lepraemurium* on supplemental minimal medium and its experimental pathogenesis. Indian J. Med. Res. **102** (1995) 104–113.

The splenic tissue of a mouse experimentally infected with *Mycobacterium lepraemurium* (Hawaiian strain, M-65) and developing "rat leprosy," yielded a pure culture of an acid-fast bacterium having all the characteristics of *M. lepraemurium* on mineral salt minimal medium supplemented with sample sources of C and N, e.g., NH_4 salts, liquid paraffin, urea, gelatin, etc. This could be maintained by serial passages *in vitro* with good growth. Its indefinite propagation with tissue-free, washed, small inoculum on complex media including Ogawa medium was difficult, and its serial subculture was practically impossible. The *in vitro* isolate from supplemented minimal medi-

um could produce pathological lesions in mice typical of rat leprosy.—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 β-lactam antibiotics be used to treat tuberculosis? Antimicrob. 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. β-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 [^{3}H]penicillin-radiolabeled membrane proteins prepared from *M. tuberculosis* H37Ra. The presence of membrane-associated β-lactamase precluded the use of membranes for assaying the binding affinities of β-lactam antibiotics. Therefore, ampicillin affinity chromatography was used to purify these four PBPs from crude membranes in order to assay the binding affinities of β-lactam antibiotics. Ampicillin, amoxicillin, and imipenem, β-lactam 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 β-lactam antibiotics to the target PBPs could not account for resistance. Resistance could be reversed with the β-lactamase inhibitors clavulanate or sulbactam or could be circumvented by the use of a β-lactamase-stable drug, imipenem, indicating that mycobacterial β-lactamase, probably in conjunction with slow penetration, is a major determinant of *M. tuberculosis* resistance to β-lactam antibiotics. These findings confirm *in vitro* data that *M. tuberculosis* is susceptible to some β-lactam antibiotics. Further evaluation of these drugs for the treatment of tuberculosis in animal models and in clinical trials is warranted.—Authors' Abstract

Clemens, D. L., Lee, B.-Y. and Horwitz, M. A. Purification, characterization, and genetic analysis of *Mycobacterium tuberculosis* urease, a potentially critical determinant of host-pathogen interaction. J. Bacteriol. **177** (1995) 5644–5652.

Mycobacterium tuberculosis urease (urea amidohydrolase [EC 3.5.1.5]) was purified and shown to contain three subunits: two small subunits, each approximately 11,000 Da, and a large subunit of 62,000 Da. The N-terminal sequences of the three subunits were homologous to those of the A, B, and C subunits, respectively, of other bacterial ureases. *M. tuberculosis* urease was specific for urea, with a K_m of 0.3 mM, and did not hydrolyze thiourea, hydroxyurea, arginine, or asparagine. The enzyme was active over a broad pH range (optimal activity at pH 7.2) and was remarkably stable against heating to 60°C and resistant to denaturation with urea. The enzyme was not inhibited by 1 mM EDTA but was inhibited by *N*-ethylmaleimide, hydroxyurea, acetohydroxamate, and phenylphosphorodiamidate. Urease activity was readily detectable in *M. tuberculosis* growing in nitrogen-rich broth, but expression increased 10-fold upon nitrogen deprivation, which is consistent with a role for the enzyme in nitrogen acquisition by the bacterium. The gene cluster encoding urease was shown to have organizational similarities to urease gene clusters of other bacteria. The nucleotide sequence of the *M. tuberculosis* urease gene cluster revealed open reading frames corresponding to the urease A, B, and C subunits, as well as to the urease accessory molecules F and G.—Authors' Abstract

Corti, S., Chevalier, J. and Cremieux, A. Intracellular accumulation of norfloxacin in *Mycobacterium smegmatis.* Antimicrob. Agents Chemother. **39** (1995) 2466–2471.

To evaluate the intracellular accumulation of norfloxacin in mycobacteria, two

methods were used with *Mycobacterium smegmatis*. A radiometric method (K. V. Cundy, C. E. Fasching, K. E. Willard, and L. R. Peterson, J. Antimicrob. Chemother. 28:391–497, 1991) was used without great modification, but the fluorometric method (P. G. S. Mortimer and L. J. V. Piddock, J. Antimicrob. Chemother. 28:639–653, 1991) was changed considerably. Indeed, adsorption of the quinolone to the bacterial surface was characterized by measuring the level of accumulation at 0°C. Taking into account the adsorption, the pH of the washing buffer was increased from 7.0 to 9.0 to improve the desorption of norfloxacin from the cell surface. Both the fluorometric method, with the technical improvement, and the radiometric method could be used to estimate the intracellular accumulation of norfloxacin, which resulted from the difference between the whole uptake measured at 37°C and the adsorption measured at 0°C. A total of 35 ng of norfloxacin per mg of cells (dry weight) penetrated into the *M. smegmatis* cell, and the steady state was achieved in 5 min. Use of inhibitors of the proton motive force revealed that transport of norfloxacin was energy independent. Thus, the same mechanisms of quinolone accumulation that occur in eubacteria seem to occur in mycobacteria, at least in *M. smegmatis*.—Authors' Abstract

Dechastellier, C., Lang, T. and Thilo, L. Phagocytic processing of the macrophage endoparasite, *Mycobacterium avium*, in comparison to phagosomes which contain *Bacillus subtilis* or latex beads. Eur. J. Cell Biol. **68** (1995) 167–182.

The intraphagosomal survival strategy of pathogenic mycobacteria was studied in bone-marrow-derived mouse macrophages. These bacteria survive inside phagosomes by interfering in an unknown manner with phagosome processing which normally would lead to digestion of the phagocytic particle in phagolysosomes. Here, phagosome processing was compared for different phagocytic particles: live *Mycobacterium avium*, degradable *Bacillus subtilis*, or indigestible latex beads. We show detailed electron microscopic morphological observations which characterize various phases of interaction between endocytic organelles and phagosomes. We measured fusion of phagosomes with early endosomes or with lysosomes by using newly internalized endocytic contents (horseradish peroxidase, HRP) and membrane marker (plasma membrane glycoconjugates labeled with [H-3]galactose via exoglycosylation). Morphometric analysis of these observations showed that the nature of the phagocytic particle affects phagosome processing: as long as particles remain undigested, maturation of phagosomes is prevented and they remain fusogenic toward early endosomes; concurrent to particle digestion, phagosome processing proceeds toward transfer of phagocytic contents to phagolysosomes which display kinetic and compositional characteristics of lysosomes. As an intact phagocytic particle, *M. avium* remains in nonmatured phagosomes which fuse with early endosomes, but not with lysosomes. Fusion with early endosomes is reduced, thereby indicating the stage where this endoparasite exerts its effect.—Authors' Abstract

Ebrahim, O., Folb, P. I., Robson, S. C. and Jacobs, P. Blunted erythropoietin response to anaemia in tuberculosis. Eur. J. Haematol. **55** (1995) 251–254.

The precise cause of the anemia that is commonly associated with severe pulmonary tuberculosis (PTB) has not been elucidated. The role of erythropoietin (Epo), the central hormone regulating red cell formation, still awaits clarification. We therefore determined serum Epo levels in patients with PTB: group 1, hemoglobin less than 110 g/L; group 2, hemoglobin greater than 110 g/L; group 3, controls, consisted of matched individuals with uncomplicated iron deficiency; group 4, healthy volunteers. Peripheral blood monocytes were obtained from patients with PTB and the controls, cultured, and the supernatant fluid (SNF) harvested. Tumor necrosis factor-alpha (TNF-α) levels were determined in the SNF, which were then added in various dilutions to a hepatocellular carcinoma cell line (HepG2) capable of regulated EPO synthesis *in vitro*. The influence of this cytokine was defined by the addition of specific neutralizing anti-TNF-α antibodies in this assay system. Patients in group 1 had significantly lower Epo levels (54 $\pm$ 11 mU/mL)

compared with those in group 3 (142 ± 41 mU/mL) ($p < 0.01$). Monocyte supernatants from patients in the anemic PTB group had markedly elevated TNF-α levels and significantly suppressed Epo output by HepG2 cells *in vitro* ($p < 0.01$). This inhibition was consistently abrogated by TNF-α antibodies. Serum Epo levels were inappropriately low in untreated PTB patients when compared with corresponding hemoglobin levels in iron-deficient controls. This blunted response could be ascribed to release of TNF-α or other cytokines by activated monocytes.—Authors' Abstract

Griffith, D. E., Brown, B. A., Girard, W. M. and Wallace, R. J. Adverse events associated with high-dose rifabutin in macrolide-containing regimens for the treatment of *Mycobacterium avium* complex lung disease. Clin. Infect. Dis. **21** (1995) 594–598.

We initiated a multidrug trial that included high-dose rifabutin for the treatment of pulmonary *Mycobacterium avium* complex (MAC) disease. Twenty-six patients received rifabutin (600 mg/d) in combination with ethambutol, streptomycin, and either clarithromycin (500 mg b.i.d.; 15 patients) or azithromycin (600 mg/d; 11 patients). Rifabutin-related adverse events occurred in 77% of patients. Fifty-eight percent of patients required a dosage adjustment or discontinuance of rifabutin therapy. The most common adverse event was a reduction in the mean total white blood cell (WBC) count, which decreased from 8600 ± 2800/mm^3 before treatment to 4500 ± 2100/mm^3 during treatment ($p = 0.0001$). Although all patients had some decrease in WBC count, only three patients (12%) required a dosage adjustment for this reason. Other common adverse events included gastrointestinal symptoms (nausea, vomiting, or diarrhea; 42%) and abnormal liver enzyme levels (12%). Eight of 11 patients (73%) with gastrointestinal symptoms, including one patient with abnormal liver enzyme levels, required a rifabutin-dosage adjustment. The most severe adverse events, always requiring an adjustment of therapy, were a diffuse polyarthralgia syndrome (19%) and anterior uveitis (8%). The latter toxicity has previously been reported to occur only in patients with AIDS and was seen only in patients who also were receiving clarithromycin. On the basis of the current findings, we recommend that rifabutin be used at a dose of 300 mg/d in multidrug regimens that include a macrolide for treatment of MAC lung disease.—Authors' Abstract

Harland, C. C., Steventon, G. B. and Marsden, J. R. Thalidomide induced neuropathy and genetic differences in drug metabolism. Eur. J. Clin. Pharmacol. **49** (1995) 1–6.

A pharmacogenetic predisposition to thalidomide-induced neuropathy has been investigated. Differences of drug metabolism were examined in 16 patients with severe orogenital ulceration, who were treated with thalidomide (≤ 200 mg/day) for 0.3–5.0 years. Eight had evidence of early peripheral neuropathy according to nerve conduction studies. Rates of C-hydroxylation, N-acetylation, and conjugation reactions with sulfate, glucuronide and glycine, were tested with the probe compounds debrisoquine, sulphadimidine, paracetamol and aspirin, respectively. Urinary drug metabolites were analyzed by high pressure liquid chromatography. Results were compared with 16 healthy age- and sex-matched volunteers.

Of the patients 6.25% and 13.3% of the controls had a poor debrisoquine hydroxylator ratio (DMR); none of the patients with neuropathy had a poor DMR as compared to 12.5% without neuropathy. Of the patients 40.0% and 35.7% of the controls were slow acetylators; 28.6% with neuropathy were slow acetylators as opposed to 50% without neuropathy. Similarly, there were no significant differences in rates of conjugation between groups. All unaffected patients were active smokers; whereas only two of those with neuropathy smoked. Cumulative dose or duration of therapy were unrelated to risk of neuropathy.

In conclusion, changes of nerve conductivity are a frequent and unpredictable adverse effect of thalidomide (≤ 200 mg/day), although smoking may have a protective action against their development. Nerve conduction studies are required before and during treatment, irrespective of the prescribed dose.—Authors' Abstract

Hernandez Pando, R., Orozco, H., Honour, J., Silva, P., Leyva, R. and Rook, G. A. W. Adrenal changes in murine pulmonary tuberculosis; a clue to pathogenesis? FEMS Immunol. Med. Microbiol. **12** (1995) 63–72.

When mice were infected with virulent *Mycobacterium tuberculosis* H37Rv by the intra-tracheal route, there was an early phase of adrenal hyperplasia, histologically resembling the adrenocorticotropic (ACTH)-driven changes seen in Cushing's disease. This was followed at 3 weeks by progressive atrophy until the weight of the adrenals was similar to 50% of that seen in control uninfected mice, in spite of the fact that the adrenals were not infected. All layers of the adrenal cortex were affected, but the medulla was normal. Electron microscope studies revealed apoptosis. The switch from adrenal hyperplasia to adrenal atrophy corresponded to onset of an IgG1 response recognizing a wide range of mycobacterial components in Western blots. Delayed-type hypersensitivity (DTH) responses were seen throughout, but differed in their sensitivity to TNF-alpha. Thus, if TNF-alpha was injected at 24 hr into DTH sites elicited during the phase of adrenal hyperplasia, there was no increment in swelling at 48 hr. However, similar injections of TNF-alpha resulted in a doubling of the swelling in DTH sites elicited during the phase of adrenal atrophy. This may be relevant to the pathogenesis of cytokine-mediated tissue damage in the human disease. If 2 months before mice received the intratracheal infection, they were pre-immunized with 1×10^7 autoclaved *M. vaccae,* a stimulus previously shown to induce a Th1 pattern of response, the early increase in adrenal weight was attenuated and delayed, and the subsequent atrophy did not occur. In sharp contrast, pre-immunization with 1×10^9 autoclaved *M. vaccae,* known to prime a mixed pattern of cytokine release (IFN-gamma and IL-4), resulted in adrenal atrophy that began within 4 days of infection, and was complete by day 14. These results suggested that the pattern of cytokine release provoked by the infection modulated the adrenal changes, perhaps in synergy with products derived from the organisms themselves. Since we have already shown that profound adrenal changes also occur in human tuberculosis, we now propose that a change somewhere in the cytokine-hypothalamo-pituitary-adrenal axis may underlie the T-cell dysfunction and immunologically mediated tissue damage in this disease.—Authors' Abstract

Hirata, T., Saito, H., Tomioka, H., Sato, K., Jidoi, J., Hosoe K. and Hidaka, T. *In vitro* and *in vivo* activities of the benzoxazinorifamycin KRM-1648 against *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **39** (1995) 2295–2303.

The *in vitro* and *in vivo* activities of a new benzoxazinorifamycin, KRM-1648 (KRM), against *Mycobacterium tuberculosis* were studied. The MIC at which 50% of the isolates are inhibited (MIC_{50}) and the MIC_{90} of KRM for 30 fresh isolates of *M. tuberculosis* measured by the BACTEC 460 TB System were 0.016 and 2 μg/ml, respectively. These values were much lower than those for rifampin (RMP), which were 4 and >128 μg/ml, respectively, and considerably lower than those for rifabutin (RBT), which were 0.125 and 8 μg/ml, respectively. A correlational analysis of the MICs of these drugs for the clinical isolates revealed the presence of cross-resistance of the organisms to KRM and either RMP or RBT although the MICs of KRM were distributed over a much lower range than were those of the other two drugs. KRM and RMP at concentrations of 1 to 10 μg/ml almost completely inhibited the bacterial growth of RMP-sensitive strains ($H_{37}Rv$, Kurono, and Fujii) of *M. tuberculosis* phagocytosed in macrophage-derived J774.1 cells. KRM was more active than RMP in inhibiting the growth of the RMP-resistant (MIC = 8 μg/ml) Kurata strain but failed to show such an effect against the RMP-resistant (MIC >128 μg/ml) Watanabe strain. When KRM was given to *M. tuberculosis*-infected mice at dosages of 5 to 20 mg/kg of body weight by gavage, once daily six times per week from day 1 after infection, it was much more efficacious than RMP against infections induced in mice by the RMP-sensitive Kurono strain, as measured by a reduction of rates of mortality, a reduction of the frequency and extent of gross lung lesions, histopathological changes in lung tissues, and a decrease in the bacterial loads in the lungs and spleens

of infected mice. KRM also displayed significant therapeutic efficacy against infection induced by the RMP-resistant Kurata strain, while neither KRM nor RMP was efficacious against infection by the RMP-resistant Watanabe strain. In the case of infection with the Kurono strain, the efficacy of the drugs in prolonging the time of survival was in the order KRM, RBT, RMP. KRM was much more efficacious than RMP, when given at 1- to 4-week intervals. These findings suggest that KRM may be useful for the clinical treatment of tuberculosis contracted through RMP-sensitive strains, even when it is administered at long intervals.—Authors' Abstract

Iwanaga, S., Ohara, N., Kariu, T., Kimura, M., Yamasaki, N. and Yamada, T. Cloning and nucleotide sequence of the gene cluster encoding ribosomal proteins S12 and S7 from *Mycobacterium bovis* BCG. Biochem. Mol. Biol. Int. **36** (1995) 209–218.

A pair of oligonucleotide primers, based on the experimentally determined amino terminal sequence of *Mycobacterium bovis* BCG ribosomal protein S12 (MboS12) and a highly conserved sequence found in all mycobacterial ribosomal S12 proteins, was used for polymerase chain reaction (PCR) with *M. bovis* genomic DNA as template. The nucleotide sequence of 338-bp fragment thus produced confirmed its origin in MboS12 gene. A 5.0 kb EcoRI fragment of *M. bovis* DNA hybridizing to this fragment was cloned. Its sequencing analysis revealed the presence of two open reading frames in the same strand. Their amino acid sequences deduced from DNA sequence showed high homology with *Escherichia coli* ribosomal proteins S12 and S7. However, the intercistronic region between S12 and S7 genes, which plays a important role for autoregulation for the str operon in *E. coli*, is completely absent in *M. bovis*.—Authors' Abstract

Klausner, J. D., Ryder, R. W., Baende, E., Lelo, U., Williame, J. C., Ngamboli, K., Perriens, J. H., Kaboto, M. and Prignot, J. *Mycobacterium tuberculosis* in household contacts of human immunodeficiency virus type 1-seropositive patients with active pulmonary tuberculosis in Kinshasa, Zaire. J. Infect. Dis. **168** (1993) 106–111.

Rates of infection with *Mycobacterium tuberculosis* were compared in Kinshasa, Zaire, in 521 household contacts of 74 human immunodeficiency virus type 1 (HIV-1)-seropositive index patients and in 692 household contacts of 95 HIV-1-seropositive index patients with sputum smear-positive pulmonary tuberculosis. No difference was noted between contacts of HIV-1-seropositive and -seronegative patients. The increasing prevalence of *M. tuberculosis* infection with increasing age was similar in household contacts of seropositive and seronegative patients; by age 16 years, 75% were purified protein derivative-positive. The similarly low rates of *M. tuberculosis* infection in household contacts of HIV-1-seropositive and -seronegative index patients with sputum smear-positive pulmonary tuberculosis indicates that HIV-1-seropositive patients with pulmonary tuberculosis are not more infectious than HIV-1-seronegative patients with pulmonary tuberculosis.—Authors' Abstract

Ladel, C. H., Blum, C., Dreher, A., Reifenberg, K. and Kaufmann, S. H. E. Protective role of gamma/delta T cells and alpha/beta T cells in tuberculosis. Eur. J. Immunol. **25** (1995) 2877–2881.

Tuberculosis is a chronic infectious disease which causes major health problems globally. Although acquired resistance crucially depends on alpha/beta lymphocytes, circumstantial evidence suggests that, in addition, gamma/delta T lymphocytes contribute to protection against tuberculosis. We have studied *Mycobacterium tuberculosis* infection in TcR-delta(−/−) or TcR-beta(−/−) gene deletion mutants which completely lack gamma/delta T cells or alpha/beta T cells, respectively. Low inocula of *M. tuberculosis* led to death of TcR-beta(−/−) mice and transient disease exacerbation in TcR-delta(−/−) mutants. Infection with higher inocula caused rapid death of TcR-delta(−/−) mice. The development of and bacterial containment in granulomatous lesions was markedly impaired in TcR-beta(−/−), and less severly affected in TcR-delta(−/−) mutants. My-

cobacteria-induced IFN-gamma production by spleen cells *in vitro* was almost abolished in TcR-beta(−/−) and virtually unaffected in TcR-delta(−/−) mice. Our data confirm the crucial role of alpha/beta T cells in protection against established tuberculosis and formally prove a protective role of gamma/delta T cells in early tuberculosis.—Authors' Abstract

Liu, J., Rosenberg, E. Y. and Nikaido, H. Fluidity of the lipid domain of cell wall from *Mycobacterium chelonae*. Proc. Natl. Acad. Sci. U.S.A. **92** (1995) 11254–11258.

The mycobacterial cell wall contains large amounts of unusual lipids, including mycolic acids that are covalently linked to the underlying arabinogalactan-peptidoglycan complex. Hydrocarbon chains of much of these lipids have been shown to be packed in a direction perpendicular to the plane of the cell surface. In this study we examined the dynamic properties of the organized lipid domains in the cell wall isolated from *Mycobacterium chelonae* grown at 30 degrees C. Differential scanning calorimetry showed that much of the lipids underwent major thermal transitions between 30°C and 65°C, that is at temperatures above the growth temperature, a result suggesting that a significant portion of the lipids existed in a structure of extremely low fluidity in the growing cells. Spin-labeled fatty acid probes were successfully inserted into the more fluid part of the cell wall. Our model of the cell wall suggests that this domain corresponds to the outermost leaflet, a conclusion reinforced by the observation that labeling of intact cells produced electron spin resonance spectra similar to those of the isolated cell wall. Use of stearate labeled at different positions showed that the fluidity within the outer leaflet increased only slightly as the nitroxide group was placed farther away from the surface. These results are consistent with the model of mycobacterial cell wall containing an asymmetric lipid bilayer, with an internal, less fluid mycolic acid leaflet and an external, more fluid leaflet composed of lipids containing shorter chain fatty acids. The presence of the low-fluidity layer will lower the permeability of the cell wall to lipophilic antibiotics and chemotherapeutic agents and may contribute to the well-known intrinsic resistance of mycobacteria to such compounds.—Authors' Abstract

Marklund, B.-I., Speert, D. P. and Stokes, R. W. Gene replacement through homologous recombination in *Mycobacterium intracellulare*. J. Bacteriol. **177** (1995) 6100–6105.

Mycobacterium intracellulare is a slow-growing pathogenic mycobacterium closely related to *M. avium*. In contrast to *M. tuberculosis* and *M. bovis* BCG, *M. intracellulare* has received little attention as a model species for studies of mycobacterial molecular biology and genetics. This study shows that *M. intracellulare* 1403 (ATCC 35761) can be transformed by electroporation with high frequencies (up to 10^6 transformants per μg of DNA), using plasmids pYT937 and pMH94 as replicative and integrative vectors, respectively. We also describe an experimental system that we used to study DNA recombination in *M. intracellulare*. First, an integrative plasmid was introduced into *M. intracellulare* 1403. A nonreplicative, nonintegrative plasmid having homology with the integrated plasmid was then introduced, and the resultant recombinants were analyzed to distinguish between events of homologous and illegitimate recombination. No illegitimate recombination occurred; in all recombinants, a single crossover between homologous regions of the two plasmids was noted. During subsequent growth of a recombinant clone, a spontaneous deletion occurred that resulted in a gene replacement on the chromosome of *M. intracellulare* 1403. The ability to construct site-specific mutations in *M. intracellulare* will provide novel insights into the biology of slow-growing mycobacteria.—Authors' Abstract

Mikusova, K., Slayden, R. A., Besra, G. S. and Brennan, P. J. Biogenesis of the mycobacterial cell wall and the site of action of ethambutol. Antimicrob. Agents Chemother. **39** (1995) 2484–2489.

The effect of ethambutol (EMB) is primarily on polymerization steps in the biosynthesis of the arabinan component of cell-wall arabinogalactan (AG) of *Mycobacteri-*

um smegmatis. Inhibition of the synthesis of the arabinan of lipoarabinomannan (LAM) occurred later, and thus in the cases of AG and LAM, the polymerization of D-arabinofuranose apparently involves separate pathways. While the synthesis of these arabinans was normal in an EMB-resistant isogeneic strain, the addition of EMB to the resistant strain resulted in partial inhibition of the synthesis of the arabinan of LAM and the emergence of a novel, truncated form of LAM, indicating partial susceptibility of the resistant gene(s) and providing a new intermediate in the LAM biosynthetic sequence. A consequence of inhibition of AG arabinan biosynthesis is the lack of new sites for mycolate attachment and, thus, the channeling of mycolate residues into a variety of free lipids which then accumulate. The primary biochemical effects of EMB can be explained by postulating separate AG and LAM pathways catalyzed by a variety of extramembranous arabinosyl transferases with various degrees of sensitivity to EMB.—Authors' Abstract

Montoro, E., Diaz, R., Vazquez, S., Laferte, J., Suarez-Mendez, R. and Valdivia, J. A. [Evaluation of an ELISA for the serodiagnosis of tuberculosis.] Rev. Cubana Med. Trop. **46** (1994) 90–93. (in Spanish)

The use of an ELISA method for the serological diagnosis of tuberculosis was assessed through the study of the presence of circulating IgG antibodies to PPD in 220 serum samples. An 82% sensibility was determined in 50 serum samples from patients with pulmonary tuberculosis, and a specificity of 95.33% in 150 serum samples from apparently healthy subjects; 20 serum samples from patients with disorders other than tuberculosis were included in the study to determine possible crossreactions.—Authors' English Summary

Newport, M., Levin, M., Blackwell, J., Shaw, M. A., Williamson, R. and Huxley, C. Evidence for exclusion of a mutation in NRAMP as the cause of familial disseminated atypical mycobacterial infection in a Maltese kindred. J. Med. Genet. **32** (1995) 904–906.

In mice, susceptibility to intracellular infections in inbred strains is controlled by a single locus, Lsh/Ity/Bcg, and the gene responsible has been cloned and designated Nramp (natural resistance associated macrophage protein). We have identified a group of related children who appear to have a single gene defect, inherited recessively, which results in increased susceptibility to mycobacterial infection. The immunological defect observed in the affected children resembles that in mice homozygous for the Lsh/Ity/Bcg susceptible allele. To test the hypothesis that a mutation in NRAMP is responsible for the immunodeficiency observed in the affected children, we have typed eight markers in the region of human 2q34-q37 where NRAMP, the human homologue of Nramp, maps. We have shown discordance with the defect in one family and the chromosomes in the three affected children have different haplotypes, making it unlikely that inheritance of an ancestral mutation in the NRAMP gene is the cause of increased mycobacterial susceptibility in this group of children.—Authors' Abstract

Nolan, C. M., Williams, D. L., Cave, M. D., Eisenach, K. D., El Hajj, H., Hooton, T. M., Thompson, R. L. and Goldberg, S. V. Evolution of rifampin resistance in human immunodeficiency virus-associated tuberculosis. Am. J. Respir. Crit. Care Med. **152** (1995) 1067–1071.

Acquired rifampin resistance without preexisting isoniazid resistance is highly unusual in patients with tuberculosis. The purpose of this report is to describe and characterize that unusual pattern of acquired drug resistance in three patients with human immunodeficiency virus (HIV) infection. The patients originally had *Mycobacterium tuberculosis* strains that were susceptible to isoniazid and rifampin. During treatment in two patients and after completion of therapy in the remaining one, each patient developed active, rifampin-resistant, isoniazid-susceptible tuberculosis. One patient subsequently developed isoniazid resistance also. Studies on patients' *M. tuberculosis* isolates using IS6110 restriction fragment length polymorphism typing and rpoB gene sequencing indicated that rifampin resistance in each patient arose during therapy by an rpoB gene mutation in the original *M. tuberculosis* isolate. Detection

of this unusual drug-resistance phenotype in three patients with HIV infection suggests that acquired rifampin resistance is somehow associated with co-infection due to HIV and tuberculosis.—Authors' Abstract

Parker, P. M., Chao, N., Nademanee, A., O'Donnell, M. R., Schmidt, G. M., Snyder, D. S., Stein, A. S., Smith, E. P., Molina, A., Stepan, D. E., Kashyap, A., Planas, I., Spielberger, R., Somlo, G., Margolin, K., Zwingenberger, K., Wilsman, K., Negri, R. S., Long, G. D., Niland, J. C., Blume, K. G. and Forman, S. J. Thalidomide as salvage therapy for chronic graft-versus-host disease. Blood **86** (1995) 3604–3609.

Thalidomide has been reported to be an effective agent for the treatment of chronic graft-versus-host disease (CGVHD). To determine the efficacy of this agent in patients with refractory CGVHD, a total of 80 patients who failed to respond to prednisone (PSE) or PSE and cyclosporine (CSA) were treated with thalidomide; 16 patients (20%) had a sustained response, 9 with a complete remission and 7 with a partial response. Twenty-nine patients (36%) had thalidomide discontinued because of side effects, which included sedation, constipation, neuritis, skin rash, and neutropenia. Side effects were reversible with drug discontinuation except for mild residual neuritis in one case. Rashes and neutropenia have not previously been reported as thalidomide side effects when used for CGVHD treatment. We conclude thalidomide is immunosuppressive and active in the treatment of CGVHD. A high incidence of reversible side effects limited dose intensity and reduced the number of patients who could benefit from treatment.—Authors' Abstract

Peterson, P. K., Hu, S. X., Sheng, W. S., Kravitz, F. H., Molitor, T. W., Chatterjee, D. and Chao, C. C. Thalidomide inhibits tumor necrosis factor-alpha production by lipopolysaccharide- and lipoarabinomannan-stimulated human microglial cells. J. Infect. Dis. **172** (1995) 1137–1140.

Tumor necrosis factor-alpha (TNF-α) is a pathogenic factor in bacterial meningitis. The effect of thalidomide on TNF-α production by microglia, the resident macrophages of the brain, was evaluated. In primary, human, fetal, microglial cell cultures stimulated with lipopolysaccharide or lipoarabinomannan, thalidomide inhibited TNF-α release in a dose-dependent manner. The inhibitory effect of thalidomide was similar to that of dexamethasone, although expression of TNF-α mRNA in microglial cells was reduced only by thalidomide. The results of this *in vitro* study suggest that thalidomide could have therapeutic potential in gram-negative bacterial and tuberculous meningitis.—Authors' Abstract

Piersimoni, C., Tortoli, E., Mascellino, M. T., Tosi, C. P., Sbaraglia, G., Mandler, F., Bistoni, F., Bornigia, S., Desio, G., Goglio, A., Iona, E., Pasticci, M. B. and Simonetti, M. T. Activity of seven antimicrobial agents, alone and in combination, against AIDS-associated isolates of *Mycobacterium avium* complex. J. Antimicrob. Chemother. **36** (1995) 497–502.

The activity of 7 antimicrobial agents (and 5 two-drug combinations and 5 three-drug combinations) was investigated against 37 clinical isolates of *Mycobacterium avium* recovered from blood cultures of AIDS patients. The susceptibility tests were performed in Middlebrook 7H12 broth using a radiometric method. MICs of amikacin, ciprofloxacin, clarithromycin, clofazimine, ethambutol, rifabutin and sparfloxacin were determined. Five antimicrobial agents were tested in combination with clarithromycin and also with clarithromycin plus amikacin to look for possible synergic activity. Synergic activity in combination with clarithromycin and with clarithromycin plus amikacin was detected for rifabutin (54% and 51% of isolates, respectively), clofazimine (38% and 35%), ethambutol (16% and 32%), ciprofloxacin (8% and 14%) and sparfloxacin (3% and 8%). No antagonism was observed. We conclude that clarithromycin is an essential component in the chemotherapy of *M. avium* complex disease.—Authors' Abstract

Porter, J. D. H. and McAdam, K. P. W. J., eds. *Tuberculosis: Back to the Future.* Chichester, U.K.: John Wiley & Sons Ltd., 1994.

This is a most excellent account of the present global problems of tuberculosis and potential readers should not be put off by the flippant title. It is the proceedings of the London School of Hygiene and Tropical Medicine Third Annual Public Health Forum held on 18–21 April 1993. All the contributions are of the highest standard. Each chapter is followed by a discussion by an expert in the field: these discussions sometimes supplement the main contribution (for example, G.A.W. Rook and J.L. Stanford enlarge on trials of *Mycobacterium vaccae* as immunotherapy following P.E.M. Fine's comprehensive chapter on immunities) are sometimes controversial, rarely are repetitive and only in one instance are unreadable.

The volume contains up-to-date accounts of epidemiology, the impact of HIV, immunities (in the plural), diagnosis with special reference to the developing world, organization and administration of therapy including an excellent discussion of logistics, preventative therapy, control strategies, multidrug resistance and future research. Tuberculosis is a world health problem of staggering proportions. The problem is growing owing to the pandemic of HIV (especially in Asia and Africa), declining economies, spreading substance abuse, and is being compounded by the emergence of multidrug resistance. The closing chapter by Keith McAdam ends on an optimistic note and draws up 10 "commitments": national programs, faster diagnosis, increasing patient compliance, increasing program compliance, education, new drugs, appropriate intervention for HIV, a new vaccine, political partnership, and commitment to the future.

This outstanding volume is essential reading for every physician and senior student with an interest and commitment to the study and control of tuberculosis and infectious diseases generally.—A. F. Fleming (Trop. Dis. Bull.)

Prestidge, R. L., Grandison, P. M., Chuk, D. W. W., Booth, R. J. and Watson, J. D. Production of the 19-kDa antigen of *Mycobacterium tuberculosis* in *Escherichia coli* and its purification. Gene **164** (1995) 129–132.

The 19-kDa antigen (19Ag) of *Mycobacterium tuberculosis* is a lipoprotein which is released from the organism during growth. In order to study the possible involvement of this antigen in the host protective response against *M. tuberculosis* infection, it would be helpful to obtain high-level production of 19Ag from a recombinant organism. We have found that overexpression of the native 19Ag gene in *Escherichia coli* or yeast leads to products which are aggregated and insoluble. By site-directed mutagenesis of the 19Ag lipoprotein leader sequence, we have generated a mutant gene which directs the production of 19Ag into the periplasmic space of *E. coli*, from where it can be easily purified in high yield. 19Ag obtained from this mutant construct lacks the lipid-modified N-terminal Cys residue found in the native 19Ag, and is not glycosylated, but is otherwise indistinguishable from 19Ag isolated from *M. tuberculosis* culture supernatant.—Authors' Abstract

Prinsloo, Y., Van Rensburg, C. E. J., Vanderwalt, R. and Anderson, R. Augmentative inhibition of lymphocyte proliferation by cyclosporin A combined with the riminophenazine compounds clofazamine and B669. Inflamm. Res. **44** (1995) 379–385.

We have investigated the effects of cyclosporin A (CsA, 3–50 ng/ml) in combination with the riminophenazine agents clofazimine and B669 (60–500 ng/ml) on the mitogen- and alloantigen-activated proliferative responses of human mononuclear leukocytes (MNL), as well as on the phospholipase A^2 and Na+, K+-adenosine triphosphatase activities of these cells. When used in combination these agents caused inhibition of the proliferative responses of both mitogen- and alloantigen-activated MNL which was at least additive. Combinations of CsA with the riminophenazines also caused augmentative activation of PLA^2 and inhibition of Na+, K+-ATPase. The inhibitory effects of these agents, both individually and in combination, on the Na+, K+-ATPase and proliferative responses of MNL were neutralized by the membrane-stabilizing, lysophospholipid complex-forming agent alpha-tocopherol (vitamin E,

20 μg/ml). These observations suggest that combinations of CsA with riminophenazines cause interactive enhancement of the activity of PLA^2 in MNL leading to lysophospholipid-mediated inactivation of Na+, K+-ATPase and consequent inhibition of the proliferative responses of these cells. In the therapeutic setting combinations of these agents may enable reduction in the dose of CsA required to achieve meaningful immunosuppression with a consequent decrease in the risk of chemotherapy-related organ toxicity.—Authors' Abstract

Rouse, D. A., Li, Z., Bai, G.-H. and Morris, S. L. Characterization of the *katG* and *inhA* genes of isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis.* Antimicrob. Agents Chemother. **39** (1995) 2472–2477.

Resistance to isoniazid in *Mycobacterium tuberculosis* has been associated with mutations in genes encoding the mycobacterial catalase-peroxidase (*katG*) and the InhA protein (*inhA*). Among the 26 isoniazid-resistant clinical isolates evaluated in this study, mutations in putative *inhA* regulatory sequences were identified in 2 catalase-positive isolates, *katG* gene alterations were detected in 20 strains, and 4 isolates had wild-type *katG* and *inhA* genes. Mutations in the *katG* gene were detected in all 11 catalase-negative isolates: one frameshift insertion, two partial gene deletions, and nine different missense mutations were identified. An arginine-to-leucine substitution at position 463 was detected in nine catalase-positive isolates. However, site-directed mutagenesis experiments demonstrated that the presence of a leucine at codon 463 did not alter the activity of the *M. tuberculosis* catalase-peroxidase and did not affect the capacity of this enzyme to restore isoniazid susceptibility to isoniazid-resistant, KatG-defective *M. smegmatis* BH1 cells. These studies further support the association between *katG* and *inhA* gene mutations and isoniazid resistance in *M. tuberculosis,* while also suggesting that other undefined mechanisms of isoniazid resistance exist.—Authors' Abstract

Springer, B., Tortoli, E., Richter, I., Grunewald, R., Rusch-Gerdes, S., Uschmann, K., Suter, F., Collins, M. D., Kroppenstedt, R. M. and Bottger, E. C. *Mycobacterium conspicuum* sp. nov., a new species isolated from disseminated infections. J. Clin. Microbiol. **33** (1995) 2805–2811.

A new type of slowly growing, nonphotochromogenic mycobacterium was recovered from two patients with disseminated disease. The growth characteristics, acid fastness, and mycolic acids were consistent with those for *Mycobacterium* species. The results of biochemical investigations, lipid analyses, and comparative 16S rRNA sequencing showed that these isolates represent a new slowly growing *Mycobacterium* species which is named *Mycobacterium conspicuum.*—Authors' Abstract

Swartz, R. P., Roecklein, J. A., Pierce, P. F. and Yeager, H. Altered *in vitro* handling of *Mycobacterium avium* complex by monocytes and serum from HIV (+) patients. Immunol. Invest. **24** (1995) 987–998.

In patients with acquired immunodeficiency syndrome (AIDS), mycobacterial diseases are leading opportunistic infections. The reasons for the peculiar propensity for disseminated infection with *Mycobacterium avium* complex (MAC) remain unclear. We have previously examined, in detail, the ability of monocytes from healthy donors to take up and kill MAC under both nonopsonic and opsonic conditions. We have now evaluated the *in vitro* ability of peripheral blood monocytes from HIV+ patients to take up and kill MAC organisms, and have discovered a reduced ability under both nonopsonic and opsonic conditions. This reduction is due to: 1) apparent defect(s) in the phagocytes themselves, and 2) substance(s) in the HIV+ serum which actively suppresses phagocyte activity.—Authors' Abstract

Totsch, M., Brommelkamp, E., Stucker, A., Fille, M., Gross, R., Wiesner, P., Schmid, K. W., Bocker, W. and Dockhorn Dworniczak, B. Identification of mycobacteria to the species level by automated restric-

tion enzyme fragment length polymorphism analysis. Virchows Arch. **427** (1995) 85–89.

An automated method for the restriction fragment length polymorphism (RFLP) analysis for the differentiation of mycobacteria to the species level is described. After polymerase chain reaction (PCR) amplification of a sequence of the gene encoding the 65-kDa surface antigen common to all mycobacteria, the product was investigated by RFLP analysis. For accurate determination of fragment sizes, the asymmetrically fluorescein-labeled PCR product was partially digested with restriction site enzymes BstEII and HaeIII. The fragments obtained were analyzed electrophoretically using an automated laser fluorescence DNA sequencer. Determination of fragment sizes revealed a deviation of ± 1 base pair (bp; 0.6%) when compared to expected sizes. The validity of this approach was confirmed by analyzing mycobacterial DNA obtained from pure cultures of *Mycobacterium tuberculosis* and alcohol-fixed smears as well as paraffin embedded sputa of patients with culture-proven tuberculosis. Additionally a diagnostic algorithm was established by investigation of cultured *M. bovis, M. bovis* bacille Calmette-Guerin, *M. avium, M. intracellular* and *M. fortuitum.* The method allows the identification of restriction enzyme sites which are only 40 bp apart. Partial restriction enzyme digestion of asymmetrically fluorescence-labeled PCR products will presumably lead to the discovery of new restriction enzyme sites.—Authors' Abstract

van Sooligen, D., Qian, L., de Haas, P. E. W., Douglas, J. T., Traore, H., Portaels, F., Qing, H. Z., Enkhsaikan, D., Nymadawa, P. and van Embden, J. D. A. Predominance of a single genotype of *Mycobacterium tuberculosis* in countries of East Asia. J. Clin. Microbiol. **33** (1995) 3234–3238.

Analysis of the population structure of *Mycobacterium tuberculosis* strains from the People's Republic of China showed that the vast majority belong to a genetically closely related group. These strains shared the majority of their IS*6110* DNA-containing restriction fragments and, also, the DNA polymorphism associated with other repetitive DNA elements, like the polymorphic GC-rich sequence and the direct repeat, was very limited. Because the majority of these strains originated from the province of Beijing, we designated this grouping the "Beijing family" of *M. tuberculosis* strains. Strains of this family were also found to dominate in neighboring countries such as Mongolia, South Korea, and Thailand; whereas a low prevalence of such strains was observed in countries on other continents. These data indicate that strains of the Beijing family recently expanded from a single ancestor which had a selective advantage. It is speculated that long-term *M. bovis* BCG vaccination may be one of the selective forces implicated in the successful spread of the Beijing genotype.—Authors' Abstract

Young, D. B. and Duncan, K. Prospects for new interventions in the treatment and prevention of mycobacterial disease. An. Rev. Microbiol. **49** (1995) 641–673.

Mycobacterium tuberculosis claims more lives each year than any other single human pathogen. Despite the availability of effective drugs, the incidence of tuberculosis is increasing in much of the developing world and has recently re-emerged as a public health problem in industrialized countries. In the first section of this chapter, current understanding of the fundamental biology of mycobacterial infection is reviewed from the perspective of development of new tools for disease control. A second section describes strategies for identification of novel antimycobacterial agents, with particular emphasis on recent progress in defining biosynthetic pathways for unique mycobacterial cell wall components. The third section focuses on current approaches to the development of new vaccine candidates consisting of live attenuated bacteria or individual antigenic subunits.—Authors' Abstract