CURRENT LITERATURE

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

General and Historical

He, R. *et al.* [Leprosy in Huber Province (China).] China Lepr. J. **12** (1996) 20–21. (in Chinese)

As early as 2400 years ago (453 B.C.) leprosy in Hubei Province had been recorded in the book *Shiji.* The first leprosary was founded in 1894 by a church. In the period 1894 to 1950 two leprosaries accepted some 1800 patients. In 1950 the Chinese government took over these leprosaries and then made up 46 leprosy control units with working personnel of 1885, including 1231 technical persons, and 33 leprosy sections in county epidemic prevention stations for controlling the disease. By the end of 1994 a total of 14,913 leprosy patients were accumulatively found, of whom 11,943 (80.08%) have been cured. At present there are 188 active patients, the incidence and prevalence decreased by 91.95% and 97.3%, respectively, 88.6% of the counties have reached the level of basic eradication of leprosy, that is, the prevalence is below 0.01 per mil and mean incidence in the last 5 years is less than 0.5/100,000. So, thorough eradication of leprosy has become quite a real aim.—Authors' English Abstract

Noordeen, S. K. Eliminating leprosy as a public health problem—is the optimism justified? World Health Forum **17** (1996) 109–118.

Systematic use of multidrug therapy has proved to be so effective that leprosy can be eliminated as a public health problem by the end of the century. However, because of the long incubation period of this disease, together with the time-lag in case detection, the factors involved in achieving and sustaining its elimination have to be very carefully defined.—Author's Abstract

Opala, J. and Boillot, F. Leprosy among the Limba: illness and healing in the context of world view. Soc. Sci. Med. **42** (1966) 3–19.

The study analyzes the traditional beliefs and practices concerning leprosy of the Limba people of Sierra Leone, who have two views of leprosy and its cause, and two varieties of stigma associated with the disease. Treatment from the leprosy control program is only sought at a relatively advanced stage of the disease because the Limba have maintained their world view which holds a person ill only when they are in severe pain or disease. The study emphasizes the importance of world view as a key to understanding patient attitudes and behavior in developing countries, and to making valid cross-cultural comparisons.—Trop. Dis. Bull.

Chemotherapy

Barbosa dos Santos, S. N. M., Grossi Araujo, M., Gomes Pinto, D. P., Andrade Patrus, O. [Agranulocytosis induced by dapsone in a patient with tuberculoid leprosy.] An. Bras. Dermatol. **71** Suppl. 1 (1996) 16–18.

Report of a case of a female patient, under treatment for tuberculoid leprosy, taking 100 mg/day of dapsone, which developed agranulocytosis and septicemia. The mechanisms involved, possible causes and the clinical picture are discussed. There should

be caution during the use of this drug, mainly during the first 12 weeks of treatment.—Authors's English Summary

Conte, J. E., Jr., Golden, J., Duncan, S., McKenna, E., Lin, E. and Zurlinden, E. Single-dose intrapulmonary pharmacokinetics of azithromycin, clarithromycin, ciprofloxacin, and cefuroxime in volunteer subjects. Antimicrob. Agents Chemother. **40** (1996) 1617–1622.

The intrapulmonary pharmacokinetics of azithromycin, clarithromycin, ciprofloxacin, and cefuroxime were studied in 68 volunteers who received single, oral doses of azithromycin (0.5 g), clarithromycin (0.5 g), ciprofloxacin (0.5 g), or cefuroxime (0.5 g). In subgroups of four subjects each, the subjects underwent bronchoscopy and bronchoalveolar lavage at timed intervals following drug administration. Drug concentrations, including those of 14-hydroxyclarithromycin (14H), were determined in serum, bronchoalveolar lavage fluid, and alveolar cells (ACs) by high-pressure liquid chromatography. Concentrations in epithelial lining fluid (ELF) were calculated by the urea diffusion method. The maximum observed concentrations (mean ± standard deviation) of azithromycin clarithromycin, 14H, ciprofloxacin, and cefuroxime in serum were 0.13 ± 0.07, 1.0 ± 0.6, 0.60 ± 0.41, 0.95 ± 0.32, and 1.1 ± 0.3 μg/ml, respectively (all at 6 hr). None of the antibiotics except clarithromycin (39.6 ± 41.1 μg/ml) was detectable in ELF at the 6-hr bronchoscopy. The movement into and persistence in cells was different for azithromycin and clarithromycin. In ACs azithromycin was not detectable at 6 hr. reached its highest concentration at 120 hr, and exhibited the greatest area under the curve (7403 μg·hr ml^{-1}). The peak concentration of clarithromycin (181 ± 94.1 μg/ml) was greater and occurred earlier (6 hr), but the area under the curve (2006 μg·hr ml^{-1}) was less than that observed for azithromycin. 14H was detectable in ACs at 6 hr (40.3 ± 5.2 μg/ml) and 12 hr (32.8 ± 57.2 μg/ml). The peak concentration of ciprofloxacin occurred at 6 hr (4.3 ± 5.2 μg/ml), and the area under the curve was 35.0 μg·hr ml^{-1}. The data indicate that after the administration of a single dose, azithromycin, clarithromycin, and ciprofloxacin penetrated into ACs in therapeutic concentrations and that only clarithromycin was present in ELF. The correlation of these kinetic observations with clinical efficacy or toxicity was not investigated and is unclear, but the data provide a basis for further kinetic and clinical studies.—Authors' Abstract

Locher, H. H., Schlunegger, H., Hartman, P. G., Angehorn, P. and Then, R. L. Antibacterial activities of epiroprim, a new dihydrofolate reductase inhibitor, alone and in combination with dapsone. Antimicrob. Agents Chemother. 40 (1996) 1376–1381.

Epiroprim (EPM; Ro 11–8958) is a new selective inhibitor of microbial dihydrofolate reductase. EPM displayed excellent activity against staphylococci, enterococci, pneumococci, and streptococci which was considerably better than that of trimethoprim (TMP). EPM was also active against TMP-resistant strains, although the MICs were still relatively high. Its combination with dapsone (DDS) was synergistic and showed an *in vitro* activity superior to that of the TMP combination with sulfamethoxazole (SMZ). The EPM-DDS (ratio, 1:19) combination inhibited more than 90% of all important gram-positive pathogens at a concentration of 2 + 38 μg/ml. Only a few highly TMP-resistant staphylococci and enterococci were not inhibited. EPM was also more active than TMP against *Moraxella catarrhalis, Neisseria meningitidis,* and *Bacteroides* spp., but it was less active than TMP against all other gram-negative bacteria tested. Atypical mycobacteria were poorly susceptible to EPM, but the combination with DDS was synergistic and active at concentrations most probably achievable in biological fluids (MICs from 0.25 + 4.75 to 4 + 76 μg/ml). EPM and the EPM-DDS combination were also highly active against experimental staphylococcal infections in a mouse septicemia model. The combination EPM-DDS has previously been shown to exhibit activity in *Pneumocystis carinii* and *Toxoplasma* models and, as shown in the present study, also shows good activity against a broad range of bacteria including

many strains resistant to TMP and TMP-SMZ.—Authors' Abstract

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

Leprosy is transmitted by dissemination of *Mycobacterium leprae* which are lodged in the nose of the patients suffering from multibacillary (MB) type of the disease. Rifampin, a potent bactericidal antileprotic drug, is given orally to the patients with a view to make the infective cases noninfective. Earlier work by us has shown that intranasal administration of rifampin helps in reducing the *M. leprae* load in the nose much faster than after conventional oral administration. In the present study, rifampin concentrations in plasma/urine/nasal wash of healthy volunteers following oral and intranasal administration were determined. Following intranasal administration, rifampin was not detectable in plasma and high concentrations were measured in the nasal wash. Following oral administration, rifampin was not detectable in the nasal wash, indicating that enough antibiotic levels are not available for clearing *M. leprae* from nose.—Authors' Abstract

Wang, K., *et al.* [Leprosy reaction during treatment with MDT and DDS.] China Lepr. J. **12** (1996) 28–29. (in Chinese)

A comparison of using MDT with DDS monotherapy showed that the frequency of leprosy reaction had no difference between both the groups, but the duration of the reaction was shorter in the MDT group. In view of the fact that there were still occurrences of the reaction and new deformity in 7 out of 31 cases 2 years after MDT, the authors suggest either to elongate the period of taking MDT or to combine prednisone with MDT in the first 6 months of treatment for those who showed a tendency to suffer from the reaction so as to prevent disability.—Authors' English Abstract

Clinical Sciences

Adachi, N. Charles Bonnet syndrome in leprosy; prevalence and clinical characteristics. Acta Psychiatr. Scand. **93** (1996) 279–281.

Charles Bonnet syndrome (CBS) is diagnosed when a visually impaired patient without any mental disorder develops visual hallucinations. A survey of patients in a National Leprosarium revealed that the point prevalence of CBS in leprosy was 0.4%. This prevalence appears to be high, as few cases with CBS have been reported. The semeiology of visual hallucinations was typical of CBS. However, the clinical features were different from previous reported cases because of the history of leprosy and associated multisensory loss. Patients with leprosy appear to be at increased risk for CBS, due to frequent eye complications combined with sensory loss, ageing, and intact intellectual functions.—Author's Abstract

Bansal, R., Garg, B. R., Adithan, C., Vasireddi, S. S., Kumar, V. J. and Chandra, D. Cortisol status in different types of leprosy. J. Dermatol. **22** (1995) 95–97.

Basal plasma cortisol levels in 12 controls and 60 patients (in Pondicherry, India) with different types of leprosy were within normal limits. They were significantly lower in multibacillary leprosy patients; this abnormality might be due to long-standing stress leading to adrenal exhaustion. The plasma cortisol level significantly increased after the ACTH (Synacthen) stimulation test in all of the varieties of leprosy tested, which suggests that the adrenal reserve is maintained in such cases.—Trop. Dis. Bull.

Brown, T. R., Kovindha, A., Wathanadilokkol, U., Piefer, A., Smith, T. and Kraft, G. H. Leprosy neuropathy: correlation of clinical and electrophysiological tests. Indian J. Lepr. **68** (1996) 1–14.

This report describes the neurological and electrophysiological examination of 35 subjects with leprosy (average duration of symptoms 3.4 years, average time since diagnosis 7.5 months). Clinical examination in the distribution of nondominant median and ulnar nerves was performed with the following clinical methods: touch sensation with 0.05 g monofilament nylon, thermal sensation with a thermal sensitivity testing device, voluntary muscle testing and nerve palpation. At least one abnormality was found in 22 ulnar and 13 median nerves (63% and 37%, respectively). Nerve palpation was the most frequent clinical abnormality, while the other methods had similar frequencies of abnormality.

Electrophysiological studies were performed on the ipsilateral side of the leprosy subjects and on 32 age-matched normal subjects. Electrophysiological responses from the leprosy subjects were evaluated by criteria established from normal subject data. Abnormal or absent responses were found in 21/35 ulnar sensory, 12/35 ulnar motor, 9/35 median sensory and 6/35 median motor responses among the leprosy subjects. The most important electrodiagnostic findings were: (i) low sensory amplitudes and (ii) drops in amplitude and NCV over the across-elbow segment of the ulnar nerve. Both clinical and nerve conduction abnormalities were positively associated with duration of leprosy symptoms.

The four clinical methods were compared for concordance with nerve conduction data by crosstabulation. The two sensory measures, monofilaments and the thermal sensitivity device, had the highest concordances. Usefulness of clinical tests for nerve damage in leprosy may vary depending on whether the purpose is for diagnosis, patient education or clinical followup.—Authors' Summary

Carpintero-Benitez, P., Logrono, C. and Collantes-Estevez, E. Enthesopathy in leprosy. J. Rheumatol. **23** (1996) 1020–1221.

Objective. To determine whether patients with leprosy have enthesopathy at the calcaneus of plantar fascia.

Methods. In a radiographic study of over 3 years, we investigated the presence and location of enthesophytes in patients with leprosy compared to healthy age- and sex-matched controls with no evidence of bone or joint disease.

Results. Calcaneus enthesopathy occurred significantly more frequently in patients with leprosy than in controls, but no difference in the location of enthesophytes was found between the three groups. Calcaneus spurs were detected more frequently in lepromatous patients than in tuberculoid patients.

Conclusion. Enthesitis is a manifestation of leprosy and may be more common in lepromatous than in tuberculoid patients.—Authors' Abstract

Croft, R. P. Active surveillance in leprosy: how useful is it? Lepr. Rev. **67** (1996) 135–140.

In this paper, active surveillance is compared with self-reporting as a method of detecting new nerve function loss in leprosy patients who have completed multidrug therapy (MDT). Five-hundred-three patients were selected according to new surveillance guidelines in one part of the Danish-Bangladesh Leprosy Mission leprosy control project working area. Surveillance coverage of 71% was achieved in a 7-month period. During this time, 10 released-from-treatment (RFT) patients from among the study group were found to have acute nerve damage requiring prednisolone treatment. Out of the l0, only 2 were detected actively; the remaining 8 self-reported.

It is concluded that health education given at RFT time is effective in motivating patients to self-report with acute nerve damage, and that the time spent on active surveillance could have been better used in other activities, i.e., case detection.

As a result of these findings, active surveillance has been abandoned in the leprosy control project.—Author's Summary

Duncan, M. E. Pregnancy and leprosy neuropathy. Indian J. Lepr. **68** (1996) 23–34.

Women with leprosy (even apparently cured) run a serious risk of deterioration in nerve function when they become pregnant. During pregnancy and lactation the woman with leprosy may suffer: relapse, reactiva-

tion and transient exacerbation maximally in late pregnancy; ENL in the first and third trimesters, continuing with nerve damage postpartum; RR maximally postpartum, even after MDT and RFT; neuritis affecting almost 50% of women in any pregnancy/lactation, in most cases as "silent" neuritis with new motor and sensory loss, even after MDT-RFT, and stocking-and-glove anesthesia even in PB women and post-MDT-RFT. Those incubating the infection develop overt disease frequently in reaction. This tragic cycle can only be stopped by a combination of: (i) leprologists and leprosy control personnel understanding the problems of leprosy in pregnant and lactating mothers; (ii) well-planned health education for leprosy patients, and both leprosy and maternal health care workers; and (iii) the highest standard of clinical supervision during pregnancy, prolonged lactation and at regular intervals during the woman's reproductive life, even after she would normally be released from surveillance after completion of MDT.—Author's Summary

Edward, V. K., Edward, S. and Shegaonkar, S. Dry skin lesions with marked hair loss in a case of BL leprosy; a case report. Lepr. Rev. **67** (1996) 141–144.

Skin lesions of leprosy that are anesthetic, well defined, limited in number and dry with significant hair loss generally fit into the paucibacillary (PB) spectrum. The bacteria index (BI) is expected to be negative or low. We have reported a case who presented with such findings but whose BI readings were high. Together with the biopsy findings the patient was classified as having borderline (BL) leprosy. The role of the skin-smear examination and the misleading nature of some clinical features are highlighted. The authors feel that skin-smear examinations should be performed on all leprosy patients at the time of diagnosis.—Authors' Summary

Egawa, K., Yukawa, T., Arakawa, S., Nakao, H., Inoue, T., Tanaka, T., Tsuda, F., Okamoto, H., Miyakawa, Y. and Mayumi, M. Infection with GB virus C in leprous patients in Japan. J. Med. Virol. **49** (1996) 110–114.

The detection of hepatitis C virus (HCV) in blood donors and patients with acute and chronic hepatitis has brought to the fore another virus or viruses which can be transmitted parenterally and induce liver disease. The RNA of a candidate virus designated GB virus C (GBV-C) was determined by the polymerase chain reaction with primers deduced from a helicase-like region in 229 leprous patients in Japan. GBV-C RNA was detected in 12 (5.2%) patients, and HCV RNA in 41 (18%); 3 patients were coinfected with GBV-C and HCV. The 9 patients infected with GBV-C alone had aminotransferase levels lower than the 3 patients with the mixed infection or the 38 patients infected with HCV only ($p < 0.001$). Sequence comparison within 100 base pairs in the helicase-like region suggested that 2, 3 and 3 patients, respectively, would have been infected with three distinct strains of GBV-C. These results indicate that patients with leprosy are at increased risk for infection not only with HCV, but also with GBV-C, and that the infection with GBV-C alone would not induce hepatic injuries as severe as HCV infection.—Authors' Abstract

Girdhar, B. K. Neuritic leprosy. Indian J. Lepr. **68** (1996) 35–42.

In conclusion, neuritic leprosy which is a defined type of leprosy is not uncommon, at least in some parts of the world and should be diagnosed wherever there is nerve thickening and associated nerve deficit only, i.e., even in the absence of other cardinal signs such as patch and AFB in the skin smear. It appears that in a proportion of patients, neuritic leprosy evolves into typical spectral disease. There is a need to critically review the subjects from a treatment point of view since these patients have a rather large but hidden load of bacilli, irrespective of the fact whether only one or more than one nerve is affected.—Author's Conclusion

Job, C. K. Nerve in reversal reaction. Indian J. Lepr. **68** (1996) 43–47.

1. Much of the nerve destruction in leprosy takes place during the reactive phase, both during ENL reaction and RR.

2. The high-risk patients expected to develop RR are borderline patients with generalized lesions (more than 10 skin lesions) and those presenting with three or more thickened nerve trunks.

3. In RR there is a sudden enhancement of already existing DTH to *M. leprae* and its antigens resulting in the release of excess quantities of TNF α, INF γ, and IL-2. The triggering mechanisms of this phenomenon are poorly understood.

4. The already existing granulomas suddenly increase considerably in size due to edema and rapid influx of lymphocytes, Langhan's and foreign body giant cells. Fragments of *M. leprae* are also present in the granuloma of some patients.

5. In RR, the acute granulomatous inflammation can produce destruction of nerves even to the extent of causing caseous necrosis of the nerve tissue and irreversible paralysis. The swelling of the nerves due to sudden increase in inflammatory cells and edema within an unyielding perineurium produce ischemia and transient paralysis.

6. With prompt administration of antiinflammatory drugs, paralysis recovers quickly, if it is of ischemic origin; but will not recover if the Schwann cells and other nerve tissues are destroyed as a result of the immune granuloma.

7. A course of corticosteroids for 6 months along with antileprosy therapy is suggested in high-risk patients as a preventive measure.

8. Further, the serious problem of continuing nerve damage after clinical cure should be urgently tackled.—Author's Summary and Conclusions

Katoch, K. Autonomic nerve affection in leprosy. Indian J. Lepr. **68** (1996) 49–54.

Leprosy has been shown to affect almost all systems of the human body and abnormalities in functions of autonomic nerves innervating various parts have been observed in several studies. In the skin and its appendages, the common changes are anhidrosis and varying degree of impaired sweat response. Signs of denervation of the iris and reduced intraocular pressure are permanent features of autonomic involvement in the eye. In the cardiac autonomic functions, rhythm disturbances have been documented by several investigators. Respiratory function test studies have shown impaired breath holding time and decreased response to cough as well as other changes, indicating blockade of vagus nerves and sympathetic plexus. Abnormal testicular pain sensation and diminished nocturnal penile tumescence provide evidence of affliction of autonomic nerves of the male genital system. Other important autonomic nervous system involvements include the nerves innervating the capillaries of the legs. These changes have been observed to be more in extensive and longstanding disease which indicates the need to study all these aspects in prospective studies, especially in the light of early institution of multidrug treatment.—Author's Summary

Kazen, R. Role of surgery of nerves in leprosy in the restoration of sensibility in hands and feet of leprosy patients. Indian J. Lepr. **68** (1996) 55–65.

It is my opinion that surgery of nerves improves sensation in selected patients with impaired sensation, and most likely prevents further deterioration in many cases. Published studies strengthen our opinion, but there is a need for larger, controlled, prospective studies with long-term follow up. The optimal timing for nerve decompression needs to be established. There is also a need for more differentiated therapy for which a clinical classification of the leprosy neuropathy has to be developed. Teams of physicians and surgeons should work jointly with patients; and also, when possible, together with immunologists and neurologists (and maybe psychiatrists or psychologists?). Newly developed, improved techniques such as refined neurography, multifrequency vibrometry, investigations of the autonomic system should be used for assessment of patients.

Since prevention is better than cure, early detection of the nerve damage at the grass root level and adequate treatment with proper monitoring of adequate steroid treatment is the highest priority in preventing nerve damage in leprosy. When this priority has been met, there will be far less cases to

consider for any form of nerve surgery, or surgery at all.—Author's Conclusions

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

Pure neuritis leprosy is a well-recognized clinical entity. Manifestations of leprosy in the pure neuritic form accounted for 179 patients out of the total 3853 leprosy patients (4.6%) attending our Poona Urban Leprosy Investigation Centre clinics. Patients with pure neuritic leprosy are prone to develop nerve damage. Eighty-seven (48.6%) of our pure neuritis patients presented with deformities. Involvement of the upper extremity and right ulnar nerve in particular was the most common clinical feature. Patients presenting with involvement of two nerves of the same extremity was also quite common. None of our patients developed skin lesions while on antileprosy treatment. It is important to recognize neuritic symptoms early and suspect leprosy even in the absence of skin lesions.—Authors' Abstract

Mishra, B., Mukherjee, A., Girdhar, A., Husain, S., Malaviya, G. N. and Girdhar, B. K. Neuritic leprosy: further progression and significance. Acta Leprol. **9** (1995) 187–194.

Sixteen neuritic cases have been seen developing cutaneous lesions. These cutaneous lesions by and large appear within 4 months after the diagnosis of neuritis leprosy. Leprosy pathology in cutaneous lesions has been found ranging between the indeterminate and borderline lepromatous group. Development of cutaneous lesions does not seem to be influenced by age, sex or number of nerves or lepromin status. Neither do lesions seem to appear in any particular part of the body. Therapy, duration and type, i.e., monodrug or multidrug, also does not seem to influence the development of cutaneous lesions in either way. It appears that neuritic cases with either very early (indeterminate) or with advanced multibacillary neural pathology may develop skin lesions. Skin lesions possibly appear following reversal reaction in skin. Cases with newly developed skin lesions respond well to standard therapy. Development of cutaneous lesions by neuritis cases possibly indicates the natural history of the disease, conforming to the hypothesis that leprosy is basically neural in inception and that all other forms emerge from it.—Authors' Summary

Naik, S. S. and More, P. R. The pattern of "drop-out" of smear-positive cases at an urban leprosy center. Indian J. Lepr. **68** (1996) 161–166.

One-hundred-nineteen smear-positive leprosy cases registered at an ubran leprosy center in Bombay in 1991 were followed for 3 years to study the "drop-out" pattern in them and to judge the utility of some corrective measures for the same. The measures included having maps showing exact location of the patient's residence, paying home visits on registration days and subsequent persuasion and counselling both at the clinic and at the residence of patients. The results were compared with "drop-out" in smear-positive cases registered at the same center in 1989, 1990, 1992 and 1993. By introduction of the special measures, the "drop-out" rate was significantly reduced from 52% (for other years) to 36% (1991). The expenses incurred for the successful recovery of "drop-out prone" patients and ensuring regularity in drug intake was Rs. 659/– per patient.

This study of the "drop-out" patient shows that there are three categories of the so-called drop-outs: (i) the false "drop-outs" (51%): these patients get transfer as per their convenience to other leprosy centers or medical services (private practitioners or consultants) within the city; (ii) "drop-outs" due to migration: the migration is forced on them due to some genuine reason, and (iii) persistent offenders: this is a group of adamant, noncooperative, or, distressed patients. For the first two categories of patients it is advisable to introduce a good referral system. For the recalcitrant defaulters, supervised short-term drug therapy will probably be the best option.—Authors' Abstract

Namisato, M., Morii, K., Asami, S., Haraya, A., Joko, S., Kawatsu, K., Izumi, S. and Ogawa, H. [Uveitis in leprosy patients.] Jpn. J. Lepr. **64** (1995) 230–235. (in Japanese)

From this immunological study in Japan of 24 dermatologically cured leprosy patients with ongoing uveitis and 22 age- and type-matched controls (all patients had been skin-smear negative for >10 years), the authors propose that insufficient chemotherapy and the consequent incomplete elimination of leprosy bacilli are the risk factors for leprous uveitis in the quiescent stage of the disease.—Trop. Dis. Bull.

Nery, J. A. C., Santos, O. L. R., de Souza, M. C. F., Machado, A. M. and Gallo, M. E. N. [Lymph node involvement in virchowian hanseniasis: a case in reaction simulating lymphogranuloma venereum.] An. Bras. Dermatol. **71** (1996) 205–208. (in Portuguese)

Clinical presentation of leprosy and its reactional exteriorization often escapes the usual pattern, and makes health professionals confuse the diagnosis. The authors present a case of leprosy in a reactional state with an uncommon lymph node onset, which evoked the differential diagnosis with lymphogranuloma venereum. Histopathological examination of an inguinal lymph node showed a great amount of acid-fast bacilli. A very quick regression of the lymph node reactional state after the introduction of thalidomide, 300 mg/day, was noticed, thus confirming the diagnosis.—Authors' English Summary

Normaznah, Y., Umi Kalthum, C., Saniah, K. and Salleh, I. Scabies among leprosy patients in the National Leprosy Control Centre, Sungai Buloh, Selangor, Malaysia. Trop. Biomed. **12** (1995) 91–93.

A total of 213 leprosy patients hospitalized in the National Leprosy Centre was examined clinically and parasitologically for scabies (caused by *Sarcoptes scabiei*). The prevalence of scabies among this group was found to be fairly low, i.e., 7% were clinically positive and 4 (26.6%) of these were confirmed parasitologically. One case of crusted or Norwegian scabies was detected in a lepromatous leprosy patient. Topical 25% benzyl benzoate emulsion for all positive patients resulted in obvious clinical improvement.—Trop. Dis. Bull.

Petro, T. S. Bullous type of reaction mimicking pemphigus in lepromatous leprosy. Indian J. Lepr. **68** (1996) 179–181.

The bullous type of reaction in leprosy is ordinarily confined to Mexico and South America, but pustular and bullous reactions in leprosy have been reported in Indian patients also. In this presentation I report a case with bullous lesions resembling pemphigus. These lesions contained clear fluid which changed color and became dark brown within 48 hours.—Author's Abstract

Sharma, P., Kar, H. K., Beena, K. R., Kaur, H. and Narayan, R. Disabilities in multibacillary leprosy patients: before, during and after multidrug therapy. Indian J. Lepr. **68** (1966) 127–130.

One-hundred-fifty-one patients (125 males and 26 females) of multibacillary leprosy (LL 88, BL 40, BB 23), registered during 1986–1992 for multidrug therapy (MDT), were analyzed with reference to their disabilities before, during and after MDT. At induction 48 (31.7%) had no disability (Gr 0), 59 (39.0%) had only peripheral anesthesia (Gr 1) and 44 (29.1%) had Gr 2 and 3 deformities with or without anesthesia. The parallel analysis of the three groups, with nearly equal duration of symptoms, revealed that new deformities developed in only a few cases during and after MDT, least in the Gr 0 group. The crude fresh deformity incidence was 59.2 per 1000 person years of observation. The rate of recovery from anesthesia was higher (64%) in Gr 1 group than that (44%) in group with Gr 2, 3 deformities. No significant difference was observed between the incidence of Gr 2 deformities developed before, during and after MDT (incidence of claw hands 9.2% before and 7.9% during and after MDT, trophic ulcers 13.9% before and 17.8% during and after MDT). Out of 19 cases which

developed motor weakness during MDT and followup, 10 (52.6%) were instances of quiet nerve paralysis. Occupational factors influenced the development of deformities but not the sex and bacterial load. Generally, the lower the grade of disability at induction of the patient for MDT, the lower the chances of new disability development and higher the chances of recovery from sensory impairments.—Authors' Abstract

Theuvenet, W. J., Miyazaki, N., Roche, P. and Shrestha, I. Cytological needle aspiration for the diagnosis of pure neural leprosy. Indian J. Lepr. **68** (1996) 109–112.

In 7 of the 11 patients suspected to be having pure neural leprosy and who have had aspiration of the affected nerve, acid-fast bacilli were found, thus strongly supporting the diagnosis of pure neural leprosy. Two of these cases have been presented in the introduction of this paper as illustrative case reports.

In none of the patients who underwent cytological aspiration of the nerve was a subsequent iatrogenic loss of motor function or sensibility found, either on the next day or after 1 week. Besides minor discomfort on palpation of the nerve at the aspirated site in three patients, and that too on the first post-operative day only, no local changes could be detected.—From the Article

van Brakel, W. H. Assessment of nerve function under field conditions and its usefulness in leprosy. Indian J. lepr. **68** (1996) 119–125.

1. Nerve function assessment is essential in the primary prevention of impairment in leprosy. An assessment of motor function and sensibility should be done every 1 or 2 months, particularly during the first year of MDT.

2. The voluntary muscle test is currently the most suitable test to evaluate motor nerve function under field conditions. Under these circumstances, using a scale with clearly distinguishable grades, such as a four-point scale: strong, weak, reduced range of movement and paralyzed, is advisable.

3. To detect early sensory impairment a graded sensory test should be used. The best instrument currently available is a set of standardized nylon monofilaments, the Semmes-Weinstein monofilaments. If suitable, straight nylon is available, these can be locally produced very cheaply. If graded nylon filaments are not (yet) available, sensibility should be tested with a ballpoint pen using a standardized technique as much as possible.—Author's Conclusions and Recommendations

Yu, J. [α-Chymotrypsin irrigation of anterior chamber and extraction of cataract in 12 cases of leprosy.] China Lepr. J. **12** (1966) 11–12. (in Chinese)

The anterior chamber of the eye in 12 persons cured of leprosy with isolation of pupil and cataract was irrigated with 0.3 ml of α-chymotrypsin in 1:1000, and one minute later rinsed repeatedly with normal saline until organized matter there was removed. Then, extraction of cataract was completed. The author points out that such an irrigation of the anterior chamber followed by rinsing with normal saline is harmless to the iris and other tissues. Follow up for 3 to 6 years showed that their vision has been improved to some extent.—Author's English Abstract

Immuno-Pathology

Ashok Kumar, S. K., Reddy, B. S. N. and Ratnakar, C. Correlation of skin and nerve histopathology in leprosy. Lepr. Rev. **67** (1996) 119–225.

Discrepancies have been noted in the histopathological findings between skin and nerve lesions of leprosy patients in some recent works. We studied concurrent skin and nerve biopsies in 27 randomly selected leprosy patients to correlate the histopathological features of skin and nerve lesions, and to assess the importance of neural histology in the classification of leprosy.

Skin and nerve biopsies were diagnostic of leprosy in 23 and 26 patients, respectively. A discrepancy was found between the two in 15 cases. Neural histology was helpful in the classification of determinate forms in 24 cases while dermal histology was significant only in 16 patients. A multibacillary nerve and paucibacillary skin picture was observed in 3 patients.

It was concluded that nerve biopsy is more informative and specific than skin biopsy in the diagnosis of leprosy and further helps to classify the patients when the skin histology is inderminate or nonspecific.—Authors' Summary

Baumgart, K. W., McKenzie, K. R., Radford, A. J., Ramshaw, I. and Britton, W. J. Immunogenicity and vaccinia vectors coexpressing the 18-kilodalton protein of *Mycobacterium leprae.* Infec.t Immun. **64** (1996) 2274–2281.

The activation of antigen-specific T lymphocytes is essential for the control of leprosy infection in humans and experimental animals. T cells recognize a variety of protein antigens from *Mycobacterium leprae,* including the 18-kDa protein, which is limited in distribution among mycobacteria and which is absent from *M. tuberculosis* and the vaccine strain, *M. bovis* BCG. Adjuvant preparations of mycobacterial protein antigens have had limited protective efficacy for experimental infections in animals. Since recombinant vectors may elicit more effective T-cell responses than adjuvant preparations, recombinant vaccinia virus (VV18) and *M. bovis* BCG (BCG18) vectors expressing the 18-kDa protein of *M. leprae* were prepared. Both VV18 and BCG18 stimulated anti-18-kDa protein antibody and lymphocyte proliferative responses. Sequential immunization with VV18 followed by BCG18 induced higher levels of specific immunoglobulin G2a antibodies than immunoglobulin G1 antibodies, in contrast to immunization with VV18 or BCG18 alone. The protective efficacy of immunization with VV18 from a challenge with BCG18 was examined in two murine models of mycobacterial infection. After intravenous challenge, mice immunized with recombinant vaccinia virus exhibited lower initial levels of replication and earlier clearance of BCG18 from their spleens than mice immunized with vaccinia virus expressing an unrelated protein. After foot pad infection in a dissemination model, there was earlier clearance of BCG18 from specifically immunized mice. However, immunization of mice with VV18 did not prevent a productive mycobacterial infection.—Authors' Abstract

Crowder, C. and Taylor, H. W. Modified Fite stain for demonstration of *Mycobacterium* species in tissue sections. J. Histotechnol. **19** (1996) 133–134.

A modified Fite carbon fuchsin stain for *Mycobacterium* species and other acid-fast bacteria is described. This procedure eliminates uneven stain differentiation and areas of excess stain deposition. Harris hematoxylin is used for the counterstain to allow better nuclear detail. This stain was found to be superior to conventional Fite staining for *Mycobacterium* species and other acid-fast bacteria that are difficult to visual and for total tissue demonstration.—Authors' Abstract

Esin, S., Batoni, G., Kallenius, G., Gaines, H., Campa, M., Svenson, S. B., Andersson, R. and Wigzell, H. Proliferation of distinct human T cell subsets in response to live, killed or soluble extracts of *Mycobacterium tuberculosis* and *Mycobacterium avium.* Clin. Exp. Immunol. **104** (1996) 419–425.

The proliferative responses of distinct cell subsets from healthy, bacille Calmette-Guerin (BCG)-vaccinated blood donors were assessed after *in vitro* stimulation with live or UV-killed *Mycobacterium tuberculosis* and *M. avium* or with soluble extracts obtained from either mycobacterial species. Proliferation of cell subsets was evaluated by flow cytometric determination of 5-bromo-2'-deoxy-uridine incorporation into DNA and simultaneous identification of surface phenotypic markers. In the presence of monocytes, the response to whole (live or killed) bacteria was characterized by a predominant proliferation of $CD4^+$ alpha $beta^+$ T cells and, to a lesser extent, of $CD8^+$ alpha $beta^+$ T cells. Proliferation of $CD8^+$

alpha beta$^+$ T cells was primarily elicited by live rather than killed bacilli ($p < 0.05$). Conversely, when soluble bacterial extracts were used as stimulators, a preferential proliferation of gamma delta$^+$ T cells, expressing predominantly V gamma 9$^+$ and V delta 2$^+$ T-cell receptor chains, was recorded. Moreover, when monocyte-depleted cell populations were directly cultured with live bacteria, a marked proportion of CD3$^-$ CD16$^+$ [natural killer (NK)] cells was detected among the responding cells. Although both alpha beta, gamma delta and NK cells have been previously shown to react with mycobacteria *in vitro,* their relative contributions to the response have been difficult to assess. Using a flow cytometric technique which allows direct identification of proliferating cells within complex cell populations, our study demonstrates significant differences in the ability of various mycobacterial antigen preparations to elicit proliferation of distinct cell subsets.—Authors' Abstract

Fernandes Pimentel, M. I., do Nascimento, H. J. and Lima Filgueria, A. [Leprosy, ultraviolet radiation and cytokines.] An. Bras. Dermatol. **71** (1996) 141–146. (in Portuguese)

The authors reviewed the probable immunosuppressor effect of ultraviolet radiation in an infectious disease, leprosy, whose agent (*Mycobacterium leprae*) is an intracellular bacillus that presents skin and peripheral nerves tropism. From the current knowledge on the functioning of cell-mediated immunity, the modulating effect that ultraviolet radiation could exert on immunological reponse to *M. leprae* was reviewed based on the role of cytokines, with emphasis in the production of interleukins 1, 6, 10 (Il-1, IL-6, IL-10) and tumor necrosis factor alpha (TNF) by the epidermis, after ultraviolet irradiation.—Authors' English Summary

Hook, S., Griffin, F., Mackintosh, C. and Buchan, G. Activation of an interleukin-4 mRNA-producing population of peripheral blood monouclear cells after infection with *Mycobacterium bovis* or vaccination with killed, but not live, BCG. Immunology **88** (1996) 269–274.

This study examines the expression of mRNA for the Th2 cytokine, interleukin-4 (IL-4). Peripheral blood mononuclear cells from deer infected with *Mycobacterium bovis* or vaccinated with live or killed *M. bovis* bacillus Calmette-Guerin (BCG) were cultured with mycobacterial antigens. IL-4 mRNA production was assayed using the polymerase chain reaction. Elevated levels of IL-4 mRNA were detected in response to at least one antigen preparation in all animals infected with *M. bovis* as compared with none of the noninfected control animals. After a primary immunization, elevated levels of IL-4 mRNA were detected in only a proportion of vaccinated animals, and this did not correlate with whether the vaccine was live BCG or killed BCG in oil. After boosting, all the animals vaccinated with killed BCG in oil exhibited elevated IL-4 mRNA production, whereas none of the animals vaccinated with live BCG showed elevated levels. The data suggest that IL-4 is turned off during the immune response to live BCG, that boosting of low-dose live BCG vaccine may be required to "imprint" this signal and that this may be important in the development of protective immunity to tuberculosis. Killed BCG in adjuvant is not protective and as with experimental infection with virulent *M. bovis* it failed to switch off the IL-4 response. IL-4 may be useful as a diagnostic tool and as an *in vitro* marker of vaccine efficacy.—Authors' Abstract

Mistry, N. F., Shetty, V. and Antia, N. H. Nerve tissue culture as an approach towards the study of onset of reactions in the nerve in leprosy—observations and hypothesis. Indian J. Lepr. **68** (1996) 67–73.

On the basis of our observations, evidence from some published information, some literature combined with some intuitive reasoning, we submit that efforts for detection of nerve damaging reactions by serodiagnosis of mycobacterial antigen is likely to be futile. It is therefore a good policy to start seeking alternative approaches to detect that elusive "influx" signal. These alternative approaches could include: analysis of factors that break the blood-nerve barrier, study of activation of markers

on cells that are reflective of processes heralding the onset of reactions, study of the role of CNS in precipitation of reactions, and study of the role of accessory cells ir nerves, particularly the fibroblasts which secrete fibronectin. An alternative form of fibronectin which binds greater than 20% of T lymphocytes has been detected in the inflamed synovium. It is possible that ongoing repair processes in the peripheral nerve characterized by a dense presence of fibroblasts and resident inflammatory cells may result in the deposition of this aberrant fibronectin which is reported to have the property of inviting lymphocytic infiltration.—From the Article

Natrajan, M., Katoch, K., Katoch, V. M. and Bharadwaj, V. P. Enhancement in the histological diagnosis of indeterminate leprosy by demonstration of mycobacterial antigens. Acta Leprol. **9** (1995) 201–207.

In a clinico-pathological study of indeterminate leprosy, 56 cases were chosen based on specified clinical criteria. Their clinical features were noted, the smears for acid-fast bacilli (AFB) were prepared from lesions, lepromin inoculation and biopsies were performed from the lesional edges. They were subsequently treated with a modified extended WHO regimen for paucibacillary leprosy. On routine hematoxylin eosin (H&E) and Fite-Faraco staining of paraffin-embedded sections, histopathological confirmation of indeterminate leprosy was observed in only 17/56 (31%) of the clinically diagnosed cases; whereas the remaining were labelled as nonspecific pathology. Histometric analysis of all H&E stained sections did not show any characteristic finding which could be considered as characteristic and discriminatory for indeterminate leprosy.

Immunoperoxidase staining for the demonstration of mycobacterial antigen by direct staining procedure using conjugated rabbit anti-BCG and an indirect three-step procedure using primary rabbit anti-BCG and avidin biotin complex was next performed on the sections exhibiting nonspecific pathology. With the direct immunoperoxidase method, antigen was demonstrable in (11/35) 31% of the cases. The more sensitive indirect method could demonstrate the presence of antigen in (21/35) 60% of the cases.

This study thus shows that demonstration of mycobacterial antigen by simple and unexpensive immunoperoxidase techniques enhances the histopathologic diagnosis of indeterminate leprosy.—Authors' Summary

Parkash, O., Kumar, V., Mukherjee, A., Sengupta, U., Malaviya, G. N. and Girdhar, B. K. Membrane attack complex in thickened cutaneous sensory nerves of leprosy patients. Acta Leprol. **9** (1996) 195–199.

Membrane attack complex (MAC) is a terminal end product produced as a result of complement activation. The deposition of MAC, in tissues, is known to have a local tissue damaging effect in several clinical conditions. Therefore, an attempt was made to demonstrate MAC in peripheral nerve biopsies collected from leprosy patients. Interestingly, we could demonstrate deposition of MAC in involved cutaneous sensory nerves from most of the lepromatous leprosy patients. Contrary to this, the majority of nerve biopsies from tuberculoid leprosy patients did not stain for MAC. Although MAC-positive sections showed reactivity for S-protein, our observations support the possibility that MAC, either acting directly or indirectly, may be implicated in nerve damage, at least, in lepromatous leprosy patients.—Authors' Summary

Ramu, G., Kartikeyan, S., Balakrishnan, S., Patil, S. A., Ramanathan, V. D. and Desikan, K. V. Histological and immunological correlates of suspected leprosy lesions. Indian J. Lepr. **68** (1996) 155–159.

Thirty-two subjects with suspected leprosy lesions were investigated to assess various modalities of sensibility and sweat function and these were correlated with immunological and histological parameters.

It was found that pain and temperatures, mediated by small unmyelinated fibers were impaired in the early lesions. Impairment of the sweat function was seen only

when one of the modalities of sensibility was also affected. Antibodies specific to a protein (35 kDa) antigen and phenolic glycolipid-I of *Mycobacterium lepra* were positive in 9 and 12 cases, respectively, while 15 of the 31 biopsies revealed the presence of mycobacterial antigens in these lesions. The implications of these findings are discussed.—Authors' Abstract

Ridley, M. J. The leprosy granuloma in nerve: summary of recent work. Indian J. Lepr. **68** (1996) 93–94.

Conclusions drawn from this work indicated the important distinction between nerve and skin tissue in the immune recognition, handling and elimination of *Mycobacterium leprae.* At one site the antigen is protected and hidden by the basement membranes; at the other site it is open. Consequently, the bacterial degradation products and mass antigen complicate the pathology of the two types of lesions somewhat differently. Secondly, these studies showed how infection might pass from damaged dermal nerve bundles to involve previously unaffected dermis.—From the Article

Russell, D. G., Dant, J. and Sturgill Koszycki, S. *Mycobacterium avium*- and *Mycobacterium tuberculosis*-containing vacuoles are dynamic, fusion-competent vesicles that are accessible to glycosphingolipids from the host cell plasmalemma. J. Immunol. **156** (1996) 4764–4773.

The vacuoles inhabited by viable *Mycobacterium avium* and *M. tuberculosis* show limited fusion with endosomal and lysosomal compartments. This ability to regulate the maturation of their phagosomal compartments and restrict their differentiation into hydrolytically active vacuoles appears to correlate with the survival of the bacilli. Data presented in this current study demonstrate tvhat despite the apparent isolation of mycobacterial vacuoles from the lysosomal network, they are dynamic, fusion-competent vesicles. Exploiting the ability of cholera toxin B subunit to bind to GM1 ganglioside on the macrophage plasmalemma, we demonstrate that these glycosphingolipids have ready access to the mycobacterial vacuoles. Entry into mycobacterial vacuoles is rapid, within 5 min of addition to the cells, and does not proceed through a brefeldin A-sensitive pathway. Furthermore, the gangliosides follow a route that differs from that taken by fluid-phase markers. TLC analysis of gangliosides isolated from *Mycobacterium*-containing vacuoles, and IgG-bead phagosomes reveal similar profiles. These data indicate that rather than being fusion incompetent, mycobacterial vacuoles are actually highly dynamic, fusion-competent vesicles that behave like an extension of the recycling endosomal apparatus.—Authors' Abstract

Shetty, V. P. and Antia, N. H. A semiquantitative analysis of bacterial load in different cell types of leprous nerves using transmission electron microscope. Indian J. Lepr. **68** (1996) 105–108.

Thirty lepromatous (BL-LL) and 25 tuberculoid (TT-BT) nerve lesions obtained from untreated cases of leprosy were scanned using a transmission electron microscope for assessing the bacterial load in different cell types. The major bulk of infection was seen in the Schwann cells of nonmyelinated fibers, in both early lepromatous and tuberculoid nerve lesions, suggesting that *M. leprae* spread mainly via the Schwann cells within the nerve.—Authors' Summary

Turk, J. L. Host parasite response in nerve in leprosy. Indian J. Lepr. **68** (1996) 113–117.

Nerve granulomas occur at all points across the leprosy spectrum. Studies have been made using experimental models in which mycobacteria were injected directly in the sciatic or posterior tibial nerve of the guninea pig. Clinical and electrophysiological studies demonstrated axonal damage which was confirmed by morphometric studies showing disrupted myelin sheaths and in places complete demyelination. Further immunohistological studies showed a complete disappearance of staining for certain neuropeptides.

The role of Schwann cells has also been investigated. Schwann cells in nerves af-

fected by mycobacterial granulomas, both experimental and in leprosy patients were not demonstrated to be MHC class II positive, suggesting that they did not play a role in antigen presentation. Macrophages in leprosy granulomas were shown to contain TNF-α, suggesting that this cytokine played a role in axonal damage. The role of mycobacterial heat-shock protein in nerve granulomas has not as yet been determined.

The localized nature of granulomas in leprosy nerves and nerves with experimental mycobacterial granulomas has been studied by a process of excision and repair with muscle grafts. Marked recovery has been demonstrated by clinical, electrophysiological, morphometric and immuno-histochemical techniques, the latter demonstrating a return of neuropeptide production.—Author's Summary

Vallishayee, R. S., Gupte, M. D., Anantharaman, D. S. and Nagaraju, B. Post-vaccination sensitization with ICRC vaccine. Indian J. Lepr. **86** (1996) 167–174.

The ICRC vaccine is one of the candidate antileprosy vaccines under test in a large-scale comparative vaccine in trial. The objectives of the present study were to study the sensitization potential, as measured by Rees' MLSA and lepromin, and reactogenicity of this vaccine preparation in the local population. The study included 368 "healthy" individuals aged 1–70 years. Each individual received either ICRC vaccine or normal saline (control) by random allocation. They were also tested with Rees' MLSA and lepromin-A 12 weeks after vaccination. Reactions to Rees' MLSA were measured after 48 hr and those to lepromin-A after 48 hr and 3 weeks. Character and size of local response, at the vaccination site, were recorded at the 3rd, 8th and 15th week after vaccination. The results of the study showed that healing of the vaccination lesion was uneventful, the mean size of the lesion being 10.3 mm. The mean sizes of post-vaccination reactions, to Rees' MLSA and lepromin (both early and late reactions) were significantly higher in the vaccine group compared to that in the normal saline group; the sensitizing effect attributable to the vaccine was of the order of 3.5 mm, 1.7 mm and 2.2 mm, respectively. In conclusion, the study has demonstrated that ICRC vaccine was "safe" and produced a significant sensitizing effect, as measured by post-vaccination sensitization to Rees' MLSA and lepromin, in the local population.—Authors' Abstract

Vemuri, N., Viera, L. M., Taneja, K. K., Gangal, S. V. and Mukherjee, R. Anti-sphingolipid antibodies in the sera of leprosy patients. Lepr. Rev. **67** (1996) 119–125.

Earlier we reported the presence of significant levels of antigalacto-cerebroside (GalC) antibodies in the sera of leprosy patients. This study corroborates the above result and also gives evidence for the presence of antibodies to the nonpolar ceramide (Cer) moiety of GalC. AntiCer antibody titers were higher as compared to antiGalC antibodies in all categories of leprosy. The specificity of antibodies directed to the Cer moiety was confirmed using Lactosyl-BSA and neutralization assays. Statistically significant and positive correlations were observed between antiGalC and antiCer antibodies. Responsiveness factors were computed using natural logarithmic transformation of the variables.—Authors' Summary

Zaheer, S. A., Mukherjee, A., Ramesh, V., Misra, R. S., Kar, H. K., Sharma, A. K., Beena, K. R., Walia, R., Mukherjee, R., Kaur, H. and Talwar, G. P. Immunotherapy benefits multibacillary leprosy patients with persistently high bacteriological index despite long-term multidrug threrapy. Immunol. Infect. Dis. **5** (1995) 115–122.

Phase II/lII immunotherapeutic trials with *Mycobacterium w* (*M. w*) vaccine, in multibacillary (MB) leprosy patients, have clearly demonstrated immunotherapeutic benefits of the vaccine, evidenced by accelerated regression of lesions, rapid bacteriological clearance, immunological upgrading and histological improvement. This communication reports the effect of this vaccine in MB cases who showed poor bacteriological clearance as evidenced by a persistence of a high bacteria index (BI) even after a prolonged course of multidrug therapy (MDT). Thirteen MB patients from New Delhi, India, having either borderline lepro-

matous or lepromatous leprosy, who had taken MDT from 18 months to 5 years without appreciable improvement in BI were enrolled. Seven received *M. w* vaccine in addition to MDT every 3 months, while 6 received MDT plus placebo injection and served as controls. All patients were followed on identical parameters: detailed clinical scoring of lesions and biopsy at 6 months, BI and lepromin testing at 3 months, and incidence of type 2 reactions, neuritis and deformities. Compliance to MDT was monitored. All 7 patients receiving the *M. w* vaccine showed an accelerated fall in BI; 5 were rendered bacteriologically negative, histological upgrading was seen in 2 patients, 5 showed conversion to lepromin positivity after 2 to 8 doses of the vaccine. These findings were in conformity with clinical status improvement. Three experienced mild-to-moderate type 2 reactions following vaccination. No patient in the control group recorded appreciable bacteriological clearance or clinical improvement, 4 had type 2 reactions, 3 had neuritis, and 2 cases had deformities.—Trop. Dis. Bull.

Microbiology

Baulard, A., Kremer, L. and Locht, C. Efficient homologous recombination in fast-growing and slow-growing mycobacteria. J. Bacteriol. **178** (1996) 3091–3098.

Although homologous recombination is a major mechanism for DNA rearrangement in most living organisms, it has been difficult to detect in slowly growing mycobacteria by a classical suicide vector approach. Among the possible reasons for this are the low levels of transformation efficiency, the relatively high levels of illegitimate recombination, and the peculiar nature of the recA gene in slowly growing mycobacteria. In this report, we present an efficient homologous recombination system for these organisms based on the use of replicative plasmids which facilitates the detection of rare recombination events. Because the proportions of recombined molecules increase over time, intraplasmid homologous recombination in *Mycobacterium smegmatis* and *M. bovis* BCG was easily selected by the reconstitution of an interrupted kanamycin resistance gene. Chromosomal integration via homologous recombination was selected by the expression of the kanamycin resistance gene under the control of a chromosomal promoter that was not present in the plasmid before recombination. This technique was termed STORE (for selection technique of recombination events). All the clones selected by STORE had undergone homologous recombination, as evidenced by PCR analyses of the kanamycin-resistant clones. This technique should be applicable to all organisms for which homologous recombination has been difficult to achieve, provided the gene of interest is expressed.—Authors' Abstract

Bisht, D., Mehrotra, J., Dhindsa, M. S., Singh, N. B. and Sinha, S. A major T-cell-inducing cytosolic 23 kDa protein antigen of the vaccine candidate *Mycobacterium habana* is superoxide dismutase. Microbiology **142** Part 6 (1996) 1375–1383.

This study describes the purification and immunochemical characterization of a major 23-kDa cytosolic protein antigen of the vaccine candidate *Mycbacterium habana* (TMC 5135). The 23-kDa protein alone was salted out from the cytosol at an ammonium sulfate saturation of 80%–95%. It represented about 1.5% of the total cytosolic protein, appeared glycosylated by staining with periodic acid/Schiff's reagent, and showed a pI of approximately 5.3. Its native molecular mass was determined as approximately 48 kDa, suggesting a homodimeric configuration. Immunoblotting with the WHO-IMMLEP/IMMTUB mAbs mc5041 and IT61 and activity staining after native PAGE established its identity as a mycobacterial superoxide dismutase (SOD) of the Fe/Mn type. The sequence of the 18 N-terminal amino acids, which also contained the binding site for mc5041, showed a close resemblance, not only with the reported deduced sequences of *M. leprae* and *M. tuberculosis* Fe/MnSODs, but also with human MnSOD. In order to study its immuno-

pathological relevance, the protein was subjected to *in vivo* and *in vitro* assays for T-cell activation. It induced, in a-dose-related manner, skin delayed hypersensitivity in guinea pigs and lymphocyte proliferation in BALB/c mice primed with *M. habana.* Most significantly, it also induced lymphocyte proliferative responses, in a manner analogous to *M. leprae,* in human subjects comprising tuberculoid leprosy patients and healthy contacts.—Authors' Abstract

Bownds, S., Kurzynski, T. A., Norden, M. A., Dufek, J. L. and Schell, R. F. Rapid susceptibility testing for nontuberculosis mycobacterial using flow cytometry. J. Clin. Mycrobiol. **34** (1996) 1386–1390.

We demonstrated previously that susceptibility testing of *Mycobacterium tuberculosis* could be accomplished within 24 hr after the organisms were incubated with antituberculosis agents by using fluorescein diacetate (FDA) staining and flow cytometry. Continued studies have now shown that assay suspensions containing *M. avium, M. fortuitum, M. gordonae,* or *M. marinum* incubated with various concentrations of ciprofloxacin, clarithromycin, erythromycin, kanamycin, rifampin, and tobramycin hydrolyzed less FDA than drug-free controls. Suspensions of treated and nontreated mycobacteria could be easily differentiated at 6 and 24 hr after the initiation of the susceptibility assays by using FDA staining and flow cytometry. In addition, multiplication of the mycobacteria was not required to discern differences between drug-free suspensions of mycobacteria and those treated with antimycobacterial agents. The flow cytometric assay is simple, reproducible, and rapid.—Authors' Abstract

Kenney, T. J. and Churchward, G. Genetic analysis of the *Mycobacterium smegmatis rpsL* promoter. J. Bacteriol. **178** (1996) 3564–3571.

The DNA sequence of the promoter region of the *Mycobacterium smegmatis* rpsL gene, which encodes the S12 ribosomal protein, was determined. Primer extension analysis and S1 nuclease protection experiments identified the 5' end of the rpsL mRNA to be 199 bp upstream of the translation initiation codon. The rpsL, promoter contained sequences upstream of this start point for transcription that were similar to the canonical hexamers found at the –10 and –35 regions of promoters recognized by E sigma(70), the major form of RNA polymerase in *Escherichia coli.* To define the promoter of the rpsL gene, DNA fragments containing the upstream region of the rpsL gene were inserted into a plasmid vector containing a promoterless xylE gene. These insertions revealed that the 200 bp of DNA sequence immediately upstream from the translation initiation codon was not essential for promoter function. In addition, 5' deletions removing all but 34 bp upstream of the transcription start point retained greater than 90% promoter activity, suggesting that the –35 hexamer was not essential for promoter activity. To determine which nucleotides were critical for promoter function, oligonucleotide-directed mutagenesis and mutagenic PCR amplification were used to produce point mutations in the region upstream of the start point of transcription. Single base substitutions in the –10 hexamer, but not in the –35 hexamer, severely reduced rpsL promoter activity *in vivo.* Within the –10 hexamer, nucleotide substitutions causing divergence from the *E. coli* sigma(70) consensus reduced promoter activity. The DNA sequence immediately upstream from the –10 hexamer contained the TGn motif described as an extended –10 region in prokaryotic promoters. Mutations in this motif, in combination with a transition at either the –38 or –37 position within the –35 hexamer, severely reduced promoter activity, indicating that in the absence of a functional –35 region, the rpsL promoter is dependent on the TGn sequence upstream front of the –10 hexamer. Comparison of the nucleotide sequence of the rpsL promoter region of *M. smegmatis* with the homologous sequences from *M. leprae, M. bovis,* and *M. tuberculosis* showed the presence in these slowly growing mycobacterial species of conserved promoter elements a similar distance upstream of the translation initiation codon of the rpsL gene, but these other mycobacterial promoters did not contain the extended –10 motif.—Authors' Abstract

Walters, S. B. and Hanna, S. A. Testing of susceptibility of *Mycobacterium tuberculosis* to isoniazid and rifampin by mycobacterium growth indicator tube method. J. Clin. Microbiol. **34** (1966) 1565–1567.

We tested isolates of *Mycobacterium tuberculosis* recovered from 117 patients for their susceptibilities to isoniazid (INH) and rifampin (RIF) by the Centers for Disease Control and Prevention's disk modification of the indirect method of proportions (MOP) test and a three-tube mycobacteria growth indicator tube (MGIT; BBL) antimycobacterial susceptibility test (AST). Sixty-seven of the *M. tuberculosis* isolates were recovered from Lowenstein-Jensen (BBL) subcultures, and 50 of the isolates were recovered from MGIT cultures of samples from various body sites. For the MGIT AST method, 0.5 ml of test organism suspension was inoculated into an MGIT with 0.1 μg of INH per ml, an MGIT with 1.0 μg of RIF per ml, and growth control MGIT. The tubes were incubated at 37°C and were examined daily. The MGIT AST results were interpreted as follows: susceptible if the tubes containing INH or RIF did not fluoresce within 2 days of the time that the positive growth control fluoresced and resistant if the tubes containing INH or RIF did fluoresce, within 2 days of the time that the positive growth control fluoresced. The mean time fluorescence for the positive growth control was 5.5 days. The two methods were in agreement for 114 of the 117 isolates from patients, while for 3 isolates there were minor discordant results.—Authors' Abstract

Wayne, L. G. and Hayes, L. G. An *in vitro* model for sequential study of shiftdown of *Mycobacterium tuberculosis* through two stages of nonreplicating persistence. Infect. Immun. **64** (1996) 2062–2069.

It was demonstrated previously that abrupt transfer of vigorously aerated cultures of *Mycobacterium tuberculosis* to anaerobic conditions resulted in their rapid death, but gradual depletion of available O_2 permitted expression of increased tolerance to anaerobiosis. Those studies used a model based on adaptation of unagitated bacilli as they settled through a self-generated O_2 gradient, but the model did not permit examination of homogeneous populations of bacilli during discrete stages in that adaptation. The present report describes a model based on culture of tubercle bacilli in deep liquid medium with very gentle stirring that keeps them in uniform dispersion while controlling the rate at which O_2 is depleted. In this model, at least two stages of nonreplicating persistence were seen. The shift into the first stage, designated NRP stage 1, occurred abruptly at a point when the declining dissolved O_2 level approached 1% saturation. This microaerophillic stage was characterized by a slow rate of increase in turbidity without a corresponding increase in the numbers of CFU or synthesis of DNA. However, a high rate of production of glycine dehydrogenase was initiated and sustained while the bacilli were in this state, and a steady ATP concentration was maintained. When the dissolved O_2 content of the culture dropped below about 0.06% saturation, the bacilli shifted down abruptly to an anaerobic stage, designated NRP stage'2, in which no further increase in turbidity was seen and the concentration of glycine dehydrogenase declined markedly. The ability of bacilli in NRP stage 2 survive anaerobically was dependent, in part, on having spent sufficient transit time in NRP stage 1. The effects of four antimicrobial agents on the bacilli depended on which of the different physiologic stages the bacilli occupied at a given time and reflected the recognized modes of action of these agents. It is suggested that the ability to shift down into one or both of the two nonreplicating stages, corresponding to microaerophilic and anaerobic persistence, is responsible for the ability of tubercle bacilli to lie dormant in the host for long periods of time, with the capacity to revive and activate disease at a later time. The model described here holds promise as a tool to help clarify events at the molecular level that permit the bacilli to persist under adverse conditions and to resume growth when conditions become favorable. The culture model presented here is also useful for screening drugs for the ability to kill tubercle bacilli in their different stages of nonreplicating persistence.—Authors' Abstract

Experimental Infections

Shetty, V. P. and Antia, N. H. Animal model for leprous neuropathy: our contribution. Indian J. Lepr. **68** (1966) 95–104.

One message that clearly comes out of our animal studies in general is that *in-situ* Schwann cell bacillation is rare and this includes armadillos with disseminated infection and nude mice. This resistance by Schwann cells toward *Mycobacterium leprae* infection *in situ* is hard to explain and extrapolate to human subjects. It was opined by Ridley (1973) and further supported by Chatterjee (1978) that the Schwann cells were not the preferred sites for *M. leprae* invasion and/or that only free and wandering Schwann cells were capable of engulfing *M. leprae.* In the intraneural experiments there was enough room for both free Schwann cells and persistence of viable *M. leprae* in the endoneurial compartment; however, Schwann cells failed to take up *M. leprae.* This is a paradox compared to human leprosy and a gap in our knowledge. Are mice/animal Schwann cells inherently different the human ones? The answer might lie in knowing what prevents *M. leprae* from getting into the Schwann cells *in situ.*—Authors' Conclusions

Zhang, W., Wang, H., Wang, H. *et al.* [Study on minimum effective dosages and inhibitory concentrations of rifampin against *M. leprae.*] Chin. J. Clin. Dermatol. **25** (1996) 75–77. (in Chinese)

Using the mouse foot pad technique, mice were infected with four strains of *Mycobacterium leprae* which derived from untreated multibacillary leprosy cases. The mice were treated once a week with three different dosages of rifampin (RMP) by gavage in order to determine drug-susceptibility, minimum effective dosages (MED) and inhibitory concentrations (MIC) of RMP. The results showed that the MED of RMP to all the strains were ≤5 mg/kg; 3 of them were 2.5 mg/kg. The MIC of RMP were different due to different strains; they were 1.5 μg/ml, 0.8 μg/ml and 0.5 μg/ml, respectively.—Authors' English Abstract

Epidemiology and Prevention

Jesudasan, K., Vijayakumaran, P., Mani Mozhi, N. and Sundar Rao, P. S. S. A leprosy survey in a taluk headquarters town and the role of dermatological services and active health education in its success—a preliminary report. Indian J. Lepr. **68** (1996) 175–178.

A report of two general surveys done in 1984 and 1987 in Gudiyatham town is presented. The first survey covered 89.2% and the second survey 82% of the population. The new case detection rate was 3.4 per 1000. The success of the survey was due to the cooperation obtained from the public, most probably because of the intense and sustained health education, combined with leprosy services integrated with a dermatology clinic.—Authors' Abstract

Shen, J., *et al.* [Following up of household contacts with seropositivity to leprosy antigens.] China Lepr. J. **12** (1996) 14–19. (in Chinese)

Two-hundred-fifty-one residents and 141 leprosy household contacts with leprosy seropositivity, and 320 residents and 115 household contacts with seronegativity were followed up in 1992 in Wenshan and Guangnan Counties, Yunnan. The result showed that 33.47% and 6.77% of residents and 30.5% and 13.5% of contacts with seropositivity were still positive to NDO and PGL-I antigens, respectively, but 12.81% and 2.19% of residents and 20.86% and 0.87% of contacts with seronegativity became positive to NDO and PGL-I, respectively. The seropositive rate in the

younger individuals was higher and the mean level of antibody in contacts with persisting positivity was the highest. The positive rate of lepromin test in contacts was significantly lower than in residents, the rate of lepromin negativity was significantly lower than in residents, and the rate of lepromin negativity was the lowest in the contacts with persisting seropositivity. One contact with persisting seropositivity and lepromin negativity was discovered to have BL leprosy in the follow up. The authors considered that persisting seropositivity with lepromin negativity for younger individuals and household contacts possibly is a high risk factor in developing leprosy clinically.—Authors' English Abstract

Shen, Y., *et al.* [Effect of MDT on 49 MB leprosy.] China Lepr. J. **12** (1966) 22–23. (in Chinese)

Since 1986 WHO's MDT regimen has been adopted in Pingdu City, Shandong. All of 49 MB cases of leprosy who used MDT had been cured in 24 to 60 months of treatment and then have been followed up for 4 to 7 years. In the period of follow up four cases died naturally; of the remaining 45 only 1 who took 24 months of MDT relapsed in the seventh year of monitoring.—Authors' English Abstract

van Brakel, W. H. and Khawas, I. B. Nerve function impairment in leprosy: an epidemiological and clinical study—Part 2: Results of steroid treatment. Lepr. Rev. **66** (1996) 104–118.

This retrospective cohort study aimed to determine the progress of sensory and motor function during and after steroid treatment, and to identify any prognostic factors for the outcome of treatment.

The study used 168 leprosy patients registered at Green Pastures Hospital, Pokhara, West Nepal, who were treated with one of four different corticosteroid regimens for impairment of nerve function.

The function of the main peripheral nerve trunks affected in leprosy was assessed with a nylon filament to test touch thresholds (TST) and a manual voluntary muscle test (VMT) to quantify muscle strength. The TST and VMT scores at 3 months after initiation of steroid treatment served as the main outcome measure. The significance of potential prognostic factors was evaluated with logistic regression.

At 3 months, the sensory and motor function of the majority of patients with "recent" impairment (= less than 6 months' duration) had improved significantly ($p < 0.01$, Wilcoxon matched pairs signed rank test). The likelihood of "good" recovery (prognosis) for both sensibility and motor function was directly related to the severity of the nerve damage at the beginning of treatment.

Although nerve function improved in 30%–84% (depending on the type of nerve) of patients, an active search for better methods of treatment and improved regimens is justified. The need for early assessment and treatment is stressed.—Authors' Summary

Yu, X., *et al.* [Immunological surveillance and prevention of relapse in leprosy.] China Lepr. J. **12** (1996) 6–11. (in Chinese)

ELISA with dried blood spot on filter paper has been used to find anti-PGL IgM antibody special to *Mycobacterium leprae* for 5 years in succession in Shandong Province; 281 persons with ELISA positivity in the first test were randomly divided into two groups: group A, including 140 persons, was only examined one time a year and group B, having 141 persons, was given WHO's MDT in 1 year besides the examination. A total of 1234 persons with negativity served as controls. In the first test, it was found that the positive rate gradually increased with TT to LL in the classification spectrum and that the shorter the time of being cured, the higher the positive rate. During the 5-year monitoring 20 relapsed cases were detected of which 16 belong to group A, making up 11.4%, 1 to group B (0.71%) and 3 to group C (0.24%). The relative risk was 47 for those with ELISA positivity who did not take MDT, but those who had taken 1 year of MDT have only a much lower risk of relapse. The authors regard the method of monitoring relapse as being worthy to be popularized.—Authors' English Abstract

Rehabilitation

Beine, A. Abductor pollicis lonqus deviation graft operation: a new procedure on the thumb in ulnar cum low median palsy for correction of subluxation of carpometacarpal (CMC) joint. Indian J. Lepr. **68** (1996) 143–148.

For correction of instability of the carpometacarpal joint (CMC joint) of the thumb in combined paralysis of ulnar and median nerves in leprosy, bone fusing procedures have been used, but they are not desirable and can oflen be avoided

A procedure analogous to the "extensor pollicis brevis deviation graft operation'' for the correction of instability of the metacarpophalangeal joint of the thumb is described here. The new procedure appears to be useful to correct and stabilize the subluxated carpometacarpal joint of the thumb actively during the use of the hand. When thumb web contracture has occurred and the passive range of movement needed for successful opponens replacement of thumb is not available, this new procedure helps to prepare such a severe deformed thumb for correction at an earlier time—Author's Abstract

Chen, S., *et al.* [A report on rehabilitation in a leprosy hospital.] China Lepr. J. **12** (1996) 12–13. (in Chinese)

Seventeen active cases and 102 persons cured of leprosy in all in Guangahou Taihe leprosy hospital were surveyed in March 1992 and showed that the disability rate was 94.1%. Since that May a medical group for directing rehabilitation has been founded, and since then 6 cases of silent neuritis were detected and healed with prednisone without any sequelae, 107 persons were taught to do self-care, 79 pairs of protective gloves and 573 pairs of protective shoes were distributed, 118 plantar ulcers in 63 cases were cured by a comprehensive therapy in 50.9%, amputation of the leg was done in 12 cases, a total of 29 amputated persons were fitted with artificial limbs, and 18 eyes with lagophthalmos, 2 dropped feet, 3 cataracts and 3 pterygia were given surgical operations. In addition, psychological management has been undertaken.—Authors' English Abstract

Ebenezer, M., Partheebarajan, S. and Solomon, S. Long-term follow-up of joint stabilization procedures in the treatment of fixed deformities of feet in leprosy. Lepr. Rev. **67** (1996) 126–134.

This retrospective study of 52 patients, who underwent joint stabilization procedures for static deformities of the feet in leprosy between 1971 and 1985, was undertaken to assess the long-term results of joint stabilization of feet for fixed deformities in leprosy. The main purpose of joint stabilization is to make the feet plantigrade for weight bearing and to make the wearing of footwear possible. Deformities corrected include varus, equinus and equino-varus. Chronic ulceration occurs repeatedly if these deformities are not corrected and leads to inevitable bone destruction and eventual amputation.—Authors' Summary

Hirzel, C., Grauwin, M.-Y., Mane, I. and Cartel, J.-L. [Results obtained by a mobile disability prevention unit at the Institut de Léprologie (Institute of Leprosy Research), Dakar.] Acta Leprol. **9** (1995) 183–186. (in French)

Of 584 leprosy patients known at the Institut de Léprologie Appliquée de Dakar because they suffered a nerve lesion with or without chronic plantar ulcer (CPU), 242 (41%) could be followed up during a mean period of time of 8.2 years (range: 5 to 10 years) by the means of the mobile disability prevention team (health education, medical care and shoe workshop). Every 2 months a visit of the patients, at their hometown, was organized, with the purpose to assess whether they could actually put into practice the foot and hand as having been trained for. At the same time, further advice and encouragement were given to the patients. Adapted footwear was brought to the patient, at a reduced fee, the foot prints and special molds having been taken during the previous visit. The local health workers were responsible for light surgical cares.

Among the 242 followed-up patients: of 107 without CPU at beginning, 90 (84%) remained so; of 135 with CPU at beginning, 57 (42%) were cured; of 135 with CPU at beginning, 74 (55%) remained stable (no worsening); the last 21, of whom 17 showed severe foot deformities but without CPU, worsened (all presented one or more CPU at the last control).

Of the 242 patients, 221 (91%) remained stable or showed substantial improvement. Therefore, it must be emphasized that careful follow up of patients is essential to insure the improvement or care of CPU as well as to prevent the onset, worsening or reappearance of CPU. Such follow up must consist of care, health education and special shoe wearing.—Authors' English Abstract

ILEP Medical Bulletin. Prevention of disability in leprosy. Lepr. Rev. **67** (1996) 68–72.

In 1995 a survey was conducted of the prevention-of-disability policy and activities for leprosy in a random sample of 200 ILEP-assisted projects. The survey findings were reviewed at a workshop of field experts in different aspects of prevention of disability who work in various geographical regions. Recommendations (approved by the ILEP Medical Commission) were drawn up for the planning, implementation and evaluation of simple and effective prevention of disability. The survey findings and the recommendations are presented in this short article.—Trop. Dis. Bull.

Oommen, P. K. Posterior tibial neurovascular decompression for restoration of plantar sweating and sensibility. Indian J. Lepr. **68** (1996) 75–82.

It is emphasized that posterior tibial neurovascular decompression along with systemic administration of steroids in adequate doses is beneficial in early acute and silent neuritis. Nerve function, particularly the autonomic and sensory modalities, usually recovers to a considerable extent. In established cases of nerve deficit of more than 1 year, neurovascular decompression, especially the vascular part of the procedure, helps to heal chronic plantar ulcers and even prevent their recurrence. External decompression should release the flexor retinaculum by removing a width of the retinaculum to prevent its reformation. Distal compression of the plantar branches also should be relieved by slitting the calcaneal bands and ensuring free passage of the plantar branches to the sole of the foot.—Author's Conclusions

Pereira, J. H., Bowden, R. E. M., Narayanakumar, T. S. and Gschmeissner, S. E. Peripheral nerve reconstruction using denatured muscle autografts for restoring protective sensation in hands and feet of leprosy patients. Indian J. Lepr. **68** (1996) 83–91.

1. Six out of nine median nerve repairs and 30 out of 32 posterior tibial nerve repairs exhibited patchy crude sensory recovery, respectively, in hands and feet, with potential to prevent secondary damage. Overall, the quality of sensory recovery appears to be maintained in some cases up to 4 years after operation.

2. There has been one graft failure possibly due to inadequate freezing of the graft. This patient has received a second graft but it is too early to assess progress. Histology of the graft showed evidence of recognizable muscle, absence of nerve fibers and gross degree of fibrosis.

3. The technique of muscle grafting has not caused any deterioration in the neurological state of the patient from the pre-operative condition.

4. Long-term assessments and a controlled trial comparing the results of denatured muscle grafting with nerve decompression are in progress.

5. There appears to be a role for the use of denatured muscle grafts in carefully selected patients. On the basis of findings in cases of traumatic nerve repairs, earlier intervention in leprosy may produce a better result.—Authors' Conclusions

Wu, X. [Forecast of endemic trend of leprosy by Grey model.] China Lepr. J. **12** (1996) 25–27. (in Chinese)

To take endemic data of leprosy in the period of 1965 to 1991 in a country as an

object, the author introduced the use of the Grey model. (1.1) for forecast of prevalence trend of leprosy. the method is a monosequential and stepped dynamic model, may be used to forecast diseases with stable endemicity and does not need typical probability distribution. It is simple and easy to use and its accuracy of forecast is good.—Author's English Abstract

Wu, X., *et al.* [A pilot test of leprosy rehabilitation in Sichuan Province (China).] China Lepr. J. **12** (1996) 24–25. (in Chinese)

Since 1990, a pilot test in rehabilitation for leprosy was implemented under support of The Leprosy Mission International in Panzhihua City, Sichuan Province. It included detection and treatment of silent neuritis, management of complicated plantar ulcer, self-care, orthopedic surgery, amputation and fitting artificial limbs, distribution of protective shoes and utensils. Up to the end of 1994, the task has been expanded with good efficacy.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Arlen, R. R. and Wells, P. G. Inhibition of thalidomide teratogenicity by acetylsalicylic acid: evidence for prostaglandin H synthase-catalyzed bioactivation of thalidomide to a teratogenic reactive intermediate. J. Pharmacol. Exp. Therapeut. **277** (1996) 1649–1658.

Thalidomide is a teratogenic sedative-hypnotic drug that is structurally similar to phenytoin, which is thought to be bioactivated by prostaglandin H synthase (PHS) and other peroxidases to a teratogenic reactive intermediate. The relevance of this mechanism to thalidomide teratogenicity was evaluated in pregnant New Zealand White rabbits treated with thalidomide at 11:00 A.M. on gestational days 8 to 11, with day 0 indicating the time when sperm were observed in the vaginal fluid. Thalidomide (7.5 mg/kg i.v.) produced mainly fetal limb anomalies analogous to those observed in humans. Thalidomide (25–200 mg/kg i.p.) produced a dose-related increase in a spectrum of fetal anomalies, and in postpartum lethality, but did not produce a reliable incidence of limb anomalies. In subsequent studies, pregnant does received the irreversible PHS inhibitor acetylsalicylic acid (ASA), 75 mg/kg i.p., or its vehicle, followed 2 hr later by thalidomide, 7.5 mg/kg i.v., or its vehicle, ASA pretreatment was remarkably embryoprotective, resulting in respective 61.2% and 61.4% decreases in thalidomide-initiated fetal limb anomalies ($p = 0.002$) and postpartum fetal lethality ($p < 0.02$), and a small but significant reduction in tflalidomide-initiated fetal weight loss. ASA alone did not produce significant embryopathy. These results show that ASA can protect the embryo from thalidomide teratogenicity, suggesting that thalidomide may be bioactivated by PHS to a teratogenic reactive intermediate.—Authors' Abstract

Azad, A. K., Sirakova, T. D., Rogers, L. M. and Kolattukudy, P. E. Targeted replacement of the mycocerosic acid synthase gene in *Mycobacterium bovis* BCG produces a mutant that lacks mycosides. Proc. Natl. Acad. Sci. U.S.A. **93** (1996) 4787–4792.

A single gene (mas) encodes the multifunctional enzyme that catalyzes the synthesis of very long chain multiple methyl branched fatty acids called mycocerosic acids that are present only in slow-growing pathogenic mycobacteria and are thought to be important for pathogenesis. To achieve a targeted disruption of mas, an internal 2-kb segment of this gene was replaced with approximately the same size hygromycin-resistance gene (hyg), such that hyg was flanked by 4.7- and 1.4-kb segments of mas. Transformation of *Mycobacterium bovis* BCG with this construct in a plasmid that cannot replicate in mycobacteria

yielded hygromycin-resistant transformants. Screening of 38 such transformants by polymerase chain reaction (PCR) revealed several transformants representing homologous recombination with single crossover and one with double crossover. With primers representing the hyg termini and those representing the mycobacterial genome segments outside that used to make the transformation construct, the double-crossover mutant yielded PCR products expected from either side of hyg. Gene replacement was further confirmed by the absence of the vector and the 2-kb segment of mas replaced by hyg from the genome of the mutant. Thin-layer and radio-gas chromatographic analyses of the lipids derived from [1-C-14]propionate showed that the mutant was incapable of synthesizing mycocerosic acids and mycosides. Thus, homologous recombination with double crossover was achieved in a slow-growing mycobacterium with an intron-containing RecA. The resulting mas-disrupted mutant should allow testing of the postulated roles of mycosides in pathogenesis.—Authors' Abstract

Bennedsen, J., Thomsen, V. O., Pfyffer, G. E., Funke, G., Feldmann, K., Beneke, A., Jenkins, P. A., Heggin-bothom, M., Fahr, A., Hengstler, M., Cleator, G., Klapper, P. and Wilkins, E. G. L. Utility of PCR in diagnosing pulmonary tuberculosis. J. Clin. Microbiol. **34** (1996) 1407–1411.

At present, the rapid diagnosis of pulmonary tuberculosis rests with microscopy. However, this technique is insensitive and many cases of pulmonary tuberculosis cannot be initially confirmed. Nucleic acid amplification techniques are extremely sensitive, but when they are applied to tuberculosis diagnosis, they have given variable results. Investigators at six centers in Europe compared a standardized polymerase chain reaction (PCR) system (Amplicor; Roche) against conventional culture methods. Defined clinical information was collected. Discrepant samples were retested, and inhibition assays and backup amplification with a separate primer pair were performed. *Mycobacterium tuberculosis* complex organisms were recovered from 654 (9.1%) of 7194 samples and 293 (7.8%) of 3738 patients. Four-hundred-fifty-two of the *M. tuberculosis* isolates from 204 patients were smear positive and culture positive. Among the culture-positive specimens, PCR had a sensitivity of 91.4% for smear-positive specimens and 60.9% for smear-negative specimens, with a specificity of 96.1%. Analysis of 254 PCR-positive, culture-negative specimens with discrepant results revealed that 130 were from patients with recently diagnosed tuberculosis and 94 represented a presumed laboratory error. Similar analysis of 118 PCR-negative, culture-positive specimens demonstrated that 27 discrepancies were due to presumed uneven aliquot distribution and 11 were due to presumed laboratory error; PCR inhibitors were detected in 8 specimens. Amplicor enables laboratories with little previous experience with nucleic acid amplification to perform PCR. Disease in more than 60% of the patients with tuberculosis with smear-negative, culture-positive specimens can be diagnosed at the time of admission, and potentially all patients with smear-positive specimens can immediately be confirmed as being infected with *M. tuberculosis,* leading to improved clinical management.—Authors' Abstract

Boggian, K., Fierz, W., Vernazza, P. L., Battegay, M., Burgisser, P., Doorly, R., Egger, M., Erb, P., Fierz, W., Flepp, M., Francioli P., Grob, P., Gruninger, U., Hirschel, B., Ledergerber, B., Luthy, R., Malinverni, R., Matter, L., Opravil, M., Paccaud, F., Perrin, L., Pichler, W., Rickenbach, M., Rutschmann, O., Vernazza, P. and von Overbeck, J. Infrequent detection of lipoarabinomannan antibodies in human immunodeficiency virus-associated mycobacterial disease. J. Clin. Microbiol. **34** (1996) 1854–1855.

A commercially available test for the serologic diagnosis of tuberculosis was evaluated for its applicability in human immunodeficiency virus (HIV)-positive patients. Antibodies to lipoarabinomannan were detectable in sera from only 9 of 85 HIV-positive patients with a confirmed diagnosis of tuberculosis. Given the low degree of sensitivity of the assay with sera from HIV-infected patients, the study does

not support the use of this serologic assay for the diagnosis of tuberculosis in HIV-infected patients.—Authors' Abstract

Butler, W. R., Haas, W. H. and Crawford, J. T. Automated DNA fingerprinting analysis of *Mycobacterium tuberculosis* using fluorescent detection of PCR products. J. Clin. Microbiol. **34** (1996) 1801–1803.

DNA fingerprints of *Mycobacterium tuberculosis* are produced by restriction fragment length polymorphism analysis of the insertion element IS6110. We modified a polymerase chain reaction (PCR)-based subtyping method, mixed-linker PCR with fluorescent-labeled IS6110-specific oligonucleotides, to demonstrate rapid, automated, and unattended electrophoretic analysis. Variation in band sizing (normally occurring with fragment mobility), an artifact of lane-to-lane and gel-to-gel differences, was controlled with an internal lane standard, resulting in accurate and precise DNA sizing. By using this method, fingerprint analysis can be performed using actual fragment length rather than estimated position analysis.—Authors' Abstract

Fine, P. E. M. Variation in protection by BCG; implication of and for heterologous immunity. Lancet **346** (1995) 1339–1345.

Besides being the world's most widely used vaccine, and being directed against the world's leading cause of infectious disease mortality, BCG is the most controversial vaccine in current use. Estimates of protection imparted by BCG against pulmonary tuberculosis vary from nil to 80%. This variability has been attributed to strain variation in BCG preparations, to genetic or nutritional differences between populations, and to environmental influences such as sunlight exposure, poor cold-chain maintenance, or exposure to environmental mycobacterial infections. Evidence accumulated to date indicates that regional differences in environmental mycobacteria are responsible for much of the variation observed between populations in the efficacy of BCG against pulmonary tuberculosis. This paper reviews the evidence, and notes its broader implications for the epidemiology and control of mycobacterial diseases as well as for other infections and vaccines.—Trop. Dis. Bull.

Khoo, K. H., Chatterjee, D., Dell, A., Morris, H. R., Brennan, P. J. and Draper, P. Novel *o*-methylated terminal glucuronic acid characterizes the polar glycopeptidolipids of *Mycobacterium habana* strain TMC 5135. J. Biol. Chem. **271** (1996) 12333–12342.

Mycobacterium "habana" strain TMC 5135, which has been proposed as a vaccine against both leprosy and tuberculosis, is considered to be a strain of serotype I of the recognized species *M. simiae.* We have now shown that each of these strains possesses characteristic polar glycopeptidolipids (GPL) which are sufficiently different to allow unequivocal strain identification. Thin-layer chromatographic analysis demonstrated that *M. habana* synthesizes a family of apolar GPLs and three distinct polar GPLs (pGPL-I to III) which exhibited migration patterns different from those of *M. simiae* serotype I (pGPL-Sim). Using a combination of chemical, mass spectrometric, and proton-NMR analyses, the GPLs from *M. habana* were determined to be based on the same generic structure as those from the *M. avium* complex, namely N-fatty acyl-D-Phe-(O-saccharide)-D-allo-Thr-D-Ala-L-alaninyl-O-monosaccharide. The de-O-acetylated apolar GPLs contain a 3-O-Me-6-deoxy-Tal attached to the allo-Thr and either a 3-O-Me-Rha or a 3,4-di-O-Me-Rha attached to the alaninol. In the pGPLs, oligosaccharides were found to be attached to the allo-Thr. The oligoglycosyl alditol reductively released from the least polar pGPL-I was fully characterized as L-Fucp alpha 1→3-(6-O-Me)-D Glcp beta 1→3-(4-O-Me)-L-Rhap alpha 1→3-L-Rhap alpha 1→2-(3-O-Me)-6-deoxy-Tal. In pGPL-II and -III, the terminal Fuc residue is further 3-O-methylated and 4-O-substituted with an additional 2,4-di-O-Me-D-GlcA and 4-O-Me-D-GlcA, respectively. The corresponding oligosaccharide from pGPL-Sim was shown to be of identical molecular weight 60 pGPL-II but terminating with a 3,4-di-O-Me-GlcA. Enzyme-

linked immunosorbent assay-based serological studies using anti-*M. habana* and anti-*M. simiae* sera against whole cells and purified pGPLs firmly established the polar GPLs as important antigens and indicated that the terminal epitopes L-Fuc-,2,4-di-O-Me-D-GlcA, and 4-O-Me-D-GlcA uniquely present in pGPL-I, -II, and -III, respectively, confer sufficient specificity for the identification of *M. habana* as a distinct serotype of *M. simiae*.—Authors' Abstract

Kloppenburg, M., Brinkman, B. M. N., de Rooij-Dijk, H. H., Miltenburg, A. M. M., Daha, M. R., Breedveld, F. C., Dijkmans, B. A. C. and Verweli, C. L. The tetracycline derivative minocycline differentially affects cytokine production by monocytes and T lymphocytes. Antimicrob. Agents Chemother. **40** (1966) 934–940.

Minocycline is a tetracycline derivative that has beneficial effects in noninfectious forms of arthritis and dermatitis. To investigate whether this effect may be attributed to interference with cytokine production, we studied the effect of minocycline on cytokine production by T cells and monocytes. Minocycline exerted an inhibitory effect on tumor necrosis factor alpha (TNF-α) and gamma interferon production by stimulated T cells; whereas the production of interleukin 6 (IL-6) remained unaffected. The effect of minocycline on TNF-α mRNA synthesis by T cells was shown to be stimulus specific. T cells stimulated by a Ca^{2+}-independent mode exhibited a decrease in TNF-α mRNA in the presence of minocycline; whereas the TNF-α mRNA level remained unaffected by minocycline when cells were stimulated in a Ca^{2+}-dependent manner. In contrast to the effect on T cells, the addition of minocycline to lipopolysaccharide-stimulated monocytes led to a dose-dependent increase in TNF-α and IL-6 production which was paralleled by an enhancement of TNF-α mRNA synthesis. These results indicate that minocycline exerts differential effects on the regulation of cytokine production by T cells and monocytes that are partly reflected at the mRNA level. Given the pleiotropic effects of minocycline, it is suggested that the immunostimulatory effect on monocytes might counteract its beneficial properties in the treatment of several forms of chronic inflammation.—Authors' Abstract

Lemaitre, N., Sougakoff, W., Truffot, C., Grosset, J. and Jarlier, V. Restriction fragment length polymorphism of *Mycobacterium tuberculosis* strains isolated from patients with recurrence of tuberculosis. Pathol. Biol. **44** (1996) 452–455.

Analysis of restriction fragment length polymorphism (RFLP), using a DNA probe directed against the insertion sequence IS6110, was applied to strains of *Mycobacterium tuberculosis* successively isolated from four patients. In order to determine the cause of recurrence in these patients, the RFLP patterns of the corresponding isolates were analyzed. The profiles obtained from the strains isolated from each of the patients were identical, thus suggesting that a relapse, rather than an exogenous reinfection with a new strain, was the cause of recurrence. The RFLP patterns of successive isolates remained unchanged during periods of time, ranging from 5 months to 7 years, and were not modified after development of rifampin resistance. These results demonstrate the stability of the polymorphism detected by the IS6110 probe. Therefore, RFLP analysis is a powerful epidemiologic tool to distinguish relapse from exogenous reinfection.—Authors' Abstract

Meyers, W. M., Tignokpa, N., Priuli, G. B. and Portaels, F. *Mycobacterium ulcerans* infection (Buruli ulcer): first reported patients in Togo. Br. J. Dermatol. **134** (1996) 1116–1121.

Mycobacterium ulcerans infection (Buruli ulcer) is the third most common mycobacterial infection of immunocompetent humans, and is an emerging disease in West Africa. We describe the first two reported patients with Buruli ulcer in Togo, establishing a geographical continuum of the disease in all countries bordering the Gulf of Guinea. The etiological agent was identified by molecular biological analysis of biopsy material. We speculate that changing environmental factors related to human habitation may influence rates of incidence of Buruli ulcer.—Authors' Abstract

Moore, D. F., Curry, J. I., Knott, C. A. and Jonas, V. Amplification of rRNA for assessment of treatment response of pulmonary tuberculosis patients during antimicrobial therapy. J. Clin. Microbiol. **34** (1996) 1745–1749.

The time course of persistence of *Mycobacterium tuberculosis* as measured by detection of rRNA, acid-fast bacillus (AFB) smear, and culture was determined for pulmonary tuberculosis patients during antimicrobial therapy. Twenty-three patients who were initially AFB smear positive and who subsequently completed a course of antimicrobial therapy were selected for the study. Sequential specimens were tested by AFB smear, culture, and rRNA amplification (Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test [MTD]). The initial diagnostic specimens of all patients were positive by culture; those of 22 patients (96%) also were positive by MTD. Overall, MTD results remained positive longer than both smear and culture results. The median times to the last positive test result were 9 days for AFB smear, 26 days for culture, and 30 days for MTD. The last positive test result was the AFB smear result in 4% of cases, the culture result in 22%, and the MTD result in 52%. Fifty-six percent of patients had a period of shedding of noncultivable *M. tuberculosis* which was detected by MTD after culture results had converted to negative. This noncultivable period lasted 7 to 245 days. All three tests became reproducibly negative before the end of therapy and remained negative during follow up for up to 1 year. These results indicate that during successful antimicrobial therapy, *M. tuberculosis* is eliminated in sputum samples as measured by amplification of rRNA, well as by AFB smear and culture. No long-term rRNA carrier state was detected. While the time course of clearance of *M. tuberculosis* measured by rRNA overall was longer than with the two traditional tests, the rRNA test results allow sensitive and precise measurement of the clearance of noncultivable *M. tuberculosis* from respiratory specimens. This attribute may allow rRNA testing to be useful in clarifying patient response to antimicrobial therapy.—Authors' Abstract

Nash, K. A. and Inderlied, C. B. Rapid detection of mutations associated with macrolide resistance in *Mycobacterium avium* complex. Antimicrob. Agents Chemother. **40** (1996) 1748–1750.

Macrolide resistance in *Mycobacterium avium* can be detected with an adaption of a commercially available RNA/RNA duplex mismatch assay (Ambion, Austin, Tex.). The sensitivity and specificity values for the assay were 100% when evaluated against 41 macrolide-resistant and -susceptible strains of *M. avium.* Resistant subpopulations of ~20% could be readily detected. The assay is simple to perform and interpret, inexpensive, and rapid (<24-hr turnaround).—Authors' Abstract

Nicholson, S., Bonecini Almeida, M. D. G., Silva, J. R. L. E., Nathan, C., Xie, Q. W., Mumford, R. Weidner, J. R., Calaycay, J., Geng, J., Boechat, N., Linhares, C., Rom, W. and Ho, J. L. Inducible nitric oxide synthase in pulmonary aveolar macrophages from patients with tuberculosis. J. Exp. Med. **183** (1996) 2293–2302.

The high-output pathway of nitric oxide production helps protect mice from infection by several pathogens, including *Mycobacterium tuberculosis.* However, based on studies of cells cultured from blood, it is controversial whether human mononuclear phagocytes can express the corresponding inducible nitric oxide synthase (iNOS; NOS2). The present study examined alveolar macrophages directly after bronchopulmonary lavage. An average of 65% of the macrophages from 11 of 11 patients with untreated, culture-positive, pulmonary tuberculosis reacted with an antibody documented herein to be monospecific for human NOS2. In contrast, a mean of 10% of bronchoalveolar lavage cells were positive from each of five clinically normal subjects. Tuberculosis patients' macrophages displayed diaphorase activity in the same proportion that they stained for NOS2, under assay conditions wherein diaphorase reaction was strictly dependent on NOS2 expression. Bronchoalveolar lavage specimens also contained NOS2 mRNA. Thus,

macrophages in the lungs of people with clinically active *M. tuberculosis* infection often express catalytically competent NOS2.—Authors' Abstract

Roche, P. W., Feng, C. G. and Britton, W. J. Human T-cell epitopes on the *Mycobacterium tuberculosis* secreted protein MPT64. Scan. J. Immunol. **43** (1996) 662–670.

Mycobacterium tuberculosis secretes a number of proteins into the extracellular environment, some of which are restricted to the *M. tuberculosis* complex. These proteins are targets for T- and B-cell immune responses in tuberculosis (TB) patients and their contacts. The authors have mapped the immunogenic regions of the MPT64 protein of *M. tuberculosis* using peripheral blood mononuclear cells (PBMC) from TB patients and a set of overlapping peptides encompassing the complete sequence of the protein. T-cell epitopes which induced proliferation or interferon-gamma (IFN-γ) release were distributed over the full length of the protein. A C-terminal region of the protein, however, contains sequences recognized in the context of multiple HLA-DR phenotypes by more than 80% of the subjects tested. The nature of the T-cell response was further investigated by generating MPT64-specific T-cell lines. These lines also identified the T-cell epitopes in the C-terminal region of the protein. On stimulation with antigen the lines secreted IFN-γ, but not interleukin 4 (IL-4). A minority of TB patients (6/32) mounted an antibody response to MPT64. Sera from half (3/6) of these identified two linear antibody binding sites. These results confirm the significance of this prqtein in the immune response to tuberculosis infection.—Authors' Abstract

Rook, G. A. W., Honour, J., Kon, O. M., Wilkinson, R. J., Davidson, R. and Shaw, R. J. Urinary adrenal steroid metabolites in tuberculosis—a new clue to pathogenesis? QJM (Oxford) **89** (1996) 333–341.

Changes in adrenal function could explain several metabolic and immunological abnormalities in tuberculosis, and adrenal changes have been documented in a murine model of the disease. We used gas chromatography and mass spectrometry to measure 24-hr urinary outputs of steroid metabolites, before (N = 10) and at intervals during (N = 9) treatment in tuberculosis patients and in controls (N = 19). Before treatment, output of cortisol derivatives and of adrenal androgens was reduced (48%, p = 0.007; and 47%, p = 0.007, respectively). However, there was a striking increase in the ratio of cortisol to cortisone metabolites, so tetrahydrocortisol levels were normal. This agrees with the normal-to-raised plasma cortisol levels commonly seen in tuberculosis, and suggests reduced activity of 11 beta-hydroxysteroid dehydrogenase, or increased activity of the reductase. There was also an increase in the ratio of 16 alpha-hydroxylated to reduced metabolites of dehydroepiandrosterone (DHEA). The changes in the ratios of cortisol to cortisone, and of DHEA to tetrahydrocortisol tended to correct themselves during treatment, but the abnormal pattern of metabolism of DHEA showed no resolution by 40 days. This may be due to further enzyme induction by rifampin. These changes are not all disease-specific, but recent information on the functions of 11 beta-hydroxysteroid dehydrogenase and DHEA metabolites in the regulation of Thl/Th2 cytokine balance suggest a role in pathogenesis.—Authors' Abstract

Sacco, R. E., Jensen, R. J., Thoen, C. O., Sander, M., Weinstock, J., Lynch, R. G. and Dailey, M. O. Cytokine secretion and adhesion molecule expression by granuloma T lymphocytes in *Mycobacterium avium* infection. Am. J. Pathol. **148** (1996) 1935–1948.

Mice experimentally infected with *Mycobacterium avium* develop a chronic disease characterized by widespread noncaseating granulomas. In this report, we describe the phenotype and cytokine secretion profile of these granuloma-infiltrating effecter T lymphocytes. In response to specific antigen, granuloma T cells and, to a lesser extent, spleen cells secrete interferon-gamma, but no interleukin-4 or -5. The im-

portance of this Th1-like response to the host was demonstrated by the massively increased bacterial load and lethal disease in interferon-gamma knockout mice. One function of localized cytokine secretion is to recruit inflammatory T cells bearing surface adhesion molecules complementary to counter-receptors on vascular endothelial cells. Granuloma T cells express high levels of these pro-inflammatory adhesion molecules but have down-regulated their expression of L-selectin (CD62L). The expression of these adhesion molecules on granuloma-infiltrating T lymphocytes would alter the migration pathway of these cells and is likely to be important in facilitating the traffic of effecter T cells to the granulomatous inflammatory site. In addition, T cells from *Schistosoma mansoni* granulomas express the same set of adhesion molecules, showing that this phenotype is not specifically dependent upon the Th1 pattern of cytokine secretion.—Authors' Abstract

Wallace, R. J., Jr., Meier, A., Brown, B. A., Zhang, Y., Sander, P., Onyl, G. O. and Bottger, E. C. Genetic basis for clarithromycin resistance among isolates of *Mycobacterium chelonae* and *Mycobacterium abscessus.* Antimicrob. Agents Chemother. **40** (1996) 1676–1681.

Resistance to clarithromycin among isolates of *Mycobacterium chelonae* and *M. abscessus* was observed in 18 of 800 (2.3%) patients tested between 1990 and 1995. Patients whose isolates were resistant had either disseminated disease or chronic lung disease, and the resistant isolates were recovered after clarithromycin monotherapy. Sequencing of the gene coding for the 23S rRNA peptidyltransferase region revealed a point mutation involving adenine at position 2058 (38%) or adenine at position 2059 (62%) in 20 of 20 relapse isolates from the first 13 patients identified. By pulsed-field gel electrophoresis or random amplified polymorphic DNA PCR, initial and relapse isolates were shown to have identical DNA patterns. *M. chelonae* and *M. abscessus* isolates were found to have only a single chromosomal copy of the rRNA operon, thus making them susceptible to single-step mutations. Thus, clarithromycin resistance in these species of rapidly growing mycobacteria relates to a point mutation in the gene coding for 23S rRNA and occurs in limited clinical situations, but was identified in almost 5% of isolates tested in 1995.—Authors' Abstract

Xiong, Y. Q., Caillon, J., Drugeon, H., Potel, G. and Baron, D. The effect of rifampicin on adaptive resistance of *Pseudomonas aeruginosa* to aminoglycosides. J. Antimicrob. Chemother. **37** (1996) 993–998.

Using the dynamic checkerboard technique, we confirmed that rifampin produces a synergistic bactericidal effect when combined with amikacin or netilmicin. Adaptive resistance was suppressed when rifampin was added to aminoglycoside after, but not during, first exposure to amikacin or netilmicin. The effect of rifampin on adaptive resistance could account for the synergy between rifampin and aminoglycosides.—Authors' Abstract