

CURRENT LITERATURE

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

Chemotherapy

Ali, S. M. K., Khan, A. K., Khaleque, A., Hussain, A. and Shahidullah, M. *Centilla asiatica* Linn (Thankuni) in the treatment of leprosy. Bangladesh Med. Res. Cl. Bull. **12** (1986) 74-77.

Centilla asiatica (Thankuni in Bangla) grows abundantly in Bangladesh. This plant has been used by Ayurvedic practitioners since ancient times for various ailments. We have conducted a trial with the preparation from leaves of the plant. Ten lepromatous leprosy patients continued the trial for a period of 1 year and were compared with another 10 cases of lepromatous leprosy treated with dapsone. Lepromatous leprosy cases treated with Thankuni showed marked clinical and bacteriological improvement.—Authors' Summary

Bahmer, F. A. and Menzel, S. [Leprosy therapy today.] Hautarzt **38** (1987) 1-3. (in German)

The rise in the incidence of dapsone-resistant leprosy requires new therapeutic approaches. Based on the recommendations of the WHO and the experiences from many countries where leprosy is endemic, a standardized treatment regimen is presented. It is based on the combination of three chemotherapeutically active substances.—Authors' English Summary

Ganapati, R., Revankar, C. R. and Pai, R. R. Three years' assessment of multidrug therapy in multibacillary cases. Indian J. Lepr. **59** (1987) 44-49.

Analysis of the bacterial index (BI) of 584 multibacillary leprosy patients who had completed multidrug therapy (MDT) as per the recommendations of World Health Organization (WHO) and Indian Association of Leprologists (IAL) showed smear conversion rates of 56% at 24 doses and 66%

at 36 doses. Taking the BI as a parameter of judgment, the results indicate distinct improvement over the performance achieved through dapsone monotherapy during an earlier period.

The IAL regimen consisting of daily initial administration of rifampin for 21 days did not show any distinct advantage over the WHO regimen.

Bacteriological decline was uniformly noticeable in all patients although in cases with high initial BI, the smear conversion rate was much less. All six patients with BI more than 5, 59 patients (70%) with BI 4 to 4.9, and 87 patients (64%) with BI 3 to 3.9 have not been rendered negative even after 3 years of treatment.

On the contrary, 17 patients whose skin smears were still positive after receiving 24 supervised doses became bacteriologically negative subsequently, and remained so even though chemotherapy was stopped. Such studies on a large number of patients for a longer period are essential to establish whether chemotherapy should necessarily be continued up to the point of negativity.—Authors' Abstract

Gupta, P. R., Prohit, S. D., Mehta, Y. R., et al. Serum and urinary rifampicin and hepatic toxicity. Indian J. Tuberc. **32** (1985) 86-90.

Fifty, 56 and 34 patients of smear-positive pulmonary tuberculosis were randomly put on three antituberculosis drug regimens (SHE, SHRA, SHRB). Of these 0, 6, and 3 patients, respectively, developed hepatitis. It was not related to age and sex of the patients. Initial serum rifampin and urinary pure and total rifampin values were also not important but initial desacetyl rifampin excretion in urine was significantly higher in those who developed hepatitis. Nine out of 25 patients who had a ratio of more than 3

between desacetyl and pure rifampin excretion in urine developed hepatitis as compared to 0 out of 65 having a ratio of less than 3 ($p < 0.001$).—(From *Excerpta Medica*)

Hassan, M., Chaumet, S., Brauner, M. and Labadie, H. [A peculiar cause of small bowel segmental narrowing: clofazimine enteritis. Report of a case.] *Ann. Radiol. (Paris)* **29** (1986) 549–552. (in French)

Segmental ileal narrowing with a nodular submucosal pattern was observed in a patient treated by clofazimine for many years. A review of the world literature enabled the authors to retrieve four other cases with abnormal radiological findings. Hypotheses are drawn from this review to explain these radiological findings on the basis of the peculiar pathway of clofazimine, a drug mainly used for the treatment of lepromatous leprosy.—Authors' English Summary

Hugon, J. and Dumas, M. [Management of leprosy.] *Sem. Hop. Paris* **62** (1986) 1941–1945. (in French)

Conventional treatments of leprosy have failed to completely eliminate the disease as a result of increasing resistance to dapsone, the most used single drug therapy, and of the many unknown factors concerning the immunology of leprosy. This situation has prompted the World Health Organization to propose a combination of three antibiotics, i.e., rifampin, dapsone, and clofazimine. However, because it is toxic and costly, this treatment is often abandoned. Effective measures against leprosy therefore rest on very early case detection by close monitoring of family contacts, until immunization enables elimination of the disease. Development of a vaccine is now a reasonable hope as the Hansen bacillus can be cultured in armadillos and the first still ongoing trials of immunization have proved satisfactory.—Authors' English Summary

Katoch, K., Ramu, G., Ramanathan, U., Sengupta, U. and Sreevatsa. Follow up of BL/LL patients on a slightly modified WHO regimen of multidrug therapy. *Indian J. Lepr.* **59** (1987) 36–43.

Fifty-six lepromatous leprosy patients with an initial average BI of 4.45 were administered once a month 600 mg of rifampin, 100 mg of clofazimine on alternate days, and 100 mg of dapsone daily. None of these patients became smear negative in 2 years, and the same regimen was continued further. Two patients have become negative in 3 years, and treatment has been stopped in them. The study indicates that highly bacilliferous LL/BL patients are likely to need 3 years or more of multidrug therapy for achieving bacterial negativity.—Authors' Abstract

Khare, A. K., Bansal, N. K. and Meena, H. S. Dapsone syndrome—a case report. *Indian J. Lepr.* **59** (1987) 106–109.

A 31-year-old male patient with lepromatous leprosy developed fever, malaise, nausea, anorexia, lymphadenopathy, hepatitis, exfoliative dermatitis, and ainhum-like lesions while on multidrug therapy comprised of dapsone, clofazimine, and rifampin. The provocation tests confirmed the dapsone to be the cause of this event.—Authors' Abstract

Kumar, B., Kaur, S. and Kaur, I. Short term combination therapy for paucibacillary leprosy—histological evaluation & follow-up study. *Indian J. Lepr.* **59** (1987) 54–62.

Sixty-eight patients with paucibacillary disease were started on various regimes of multidrug therapy, consisting of ethionamide, rifampin, or clofazimine administered with dapsone. Serial skin biopsies were taken from 32 patients at 1, 2, and 3 years, and even later, after the initial pretreatment biopsy. Actual material was available for study from 9 patients. All regimens were tolerated well except the one with ethionamide. However, the therapeutic response was equal in all combination therapies as supported by histopathology. Compared to that with dapsone monotherapy, the response was quicker with the combination. Dapsone plus rifampin combination was best tolerated, and it worked out to be economical as well. No relapse was noted in any group during 2 or more years follow up.—Authors' Abstract

Kumar, B., Kaur, S., Kaur, I. and Gangowar, D. N. More about clofazimine—3 years' experience and review of literature. *Indian J. Lepr.* **59** (1987) 63–74.

Local and systemic side effects of clofazimine in 514 leprosy and 26 vitiligo patients who had taken the drug in different doses (100 mg to 300 mg daily) for variable periods of time. The commonest side effect noted was reddish-brown pigmentation of skin in 77.8% of the patients. In an equal number of patients, ichthyotic changes on the peripheral parts of the body were noticed. GI symptoms occurred only in 0.04% of the patients in the form of abdominal pain, epigastric distress, mild transient nausea, and anorexia. Other minor side effects noted were reddish coloration of sweat, urine, and tears. Schilling's d-xylose tests and fecal fat excretion were near normal in the 21 patients in whom these parameters were done. No abnormality in the jejunal mucosal biopsy was observed after therapy. No abnormality in the EKG or serum biochemistry occurred even after prolonged therapy. We found the drug to be very safe in the usual doses.—Authors' Abstract

Leguizamón, O. R. [Program for the eradication of leprosy; practical aspects.] *Fon-tilles Rev. Lepr.* **16** (1987) 23–31. (in Spanish)

The past 6 years since the initiation of the program for the eradication of leprosy in Paraguay with the use of multidrug therapy (MDT) (isoprodian-rifampin daily, except on Sundays) have demonstrated the effectiveness, tolerance, and extraordinary acceptability of this drug combination. Gastric side effects (14% in the first stage of the project) and incidence of hepatitis (2% in the first stage) are now less frequent. In relation to the incidence of reactions, apparently there is no difference from administering monotherapy. This should not be surprising since chemotherapy does not directly influence immunological reaction. In severe reactions, such as erythema nodosum leprosum, we administer thalidomide without discontinuing MDT. During the first 6 months, a significant decrease in the bacterial index (BI) is noticed. Chemotherapy is stopped when the BI is still positive, if

there is a good correlation with the decrease in the number of bacteria, concentration of granular forms, and general clinical picture. During the XII International Leprosy Congress, the work group "Control of Leprosy" suggested that the following parameters be used to evaluate a chemotherapeutic system: rate of relapse, incidence of reactions, side effects, effectiveness of drugs and their application under field conditions. Our system of isoprodian-rifampin is in total agreement with these requirements. During these last 6 years, we think we have initiated the correct path toward the eradication of leprosy in our country.—Authors' English Summary

McNair, A. N., Revankar, C. R. and Ganapati, R. Clinical, bacteriological and histopathological assessment of multibacillary leprosy cases after 1 and 2 years' multidrug therapy; preliminary communication. *Lepr. Rev.* **58** (1987) 182–186.

Seventy-three multibacillary leprosy patients in Bombay, India, who had completed 12 and 24 monthly doses of multidrug therapy (MDT) were subjected to clinical, bacteriological, and histopathological assessment. Only 1 in 17 of the 12-dose group with initial bacterial index (BI) ≥ 2 was rendered smear negative. This increased to more than 50% in the 24-dose group with the same BI. A number of discrepancies were noted in the correlation between clinical, bacteriological, and histopathological findings in treated cases in both the 12- and the 24-month groups and the possible reasons are discussed. This report is preliminary and long-term studies on a larger scale are clearly needed for more accurate assessment, but the data so far available suggest that considerable emphasis should be given to the BI, as the least subjective test, in deciding when to stop chemotherapy.—Authors' Summary

Ramanan, R., Manglani, P. R., Ghorpade, A. and Bhagoliwal, S. K. Follow-up study of paucibacillary leprosy on multidrug regimen. *Indian J. Lepr.* **59** (1987) 50–53.

One hundred twenty-nine registered cases of paucibacillary leprosy were put on dapsone daily and rifampin once a month and

were followed up for 1 year. Out of 129 cases, 108 (83.7%) were found to be clinically active at the end of 1 year of multidrug treatment (MDT). In 25 out of these 108 cases, a skin biopsy was done and well-defined granulomas were seen after therapy in 11 patients (44%).—Authors' Abstract

Shangluo Sanatorium. [Treatment of multibacillary leprosy with MDT for half a year.] *China J. Lepr.* **1** (1987) 11–12. (in Chinese)

Twenty-six cases of multibacillary leprosy have been treated with multidrug regimens consisting of dapsone and rifampin or dapsone, rifampin, and clofazimine for half a year. The authors think the regimens are safe and effective and enable the bacterial index to decrease more rapidly and have no toxic effects on the liver.—Authors' English Abstract

Van Assendelft, A. H. W. Renal failure and haemolysis caused by rifampicin. *Tubercle* **67** (1986) 234–235.

A 50-year-old woman was prescribed 600 mg rifampin daily for tuberculosis. Three and a half years earlier she had received 9 months' chemotherapy including rifampin, during which she developed leukopenia. The patient had for some years been treated with sodium aurothiomalate and prednisolone (2.5 mg) for rheumatoid arthritis and myalgia. After starting rifampin her myalgia worsened and the prednisolone dose was increased to 5 mg on the sixth day. On the seventh day within 1 hr of taking rifampin she developed a "flu" syndrome. On the seventh day within 1 hr of taking the drug she suffered hemolysis, icterus and renal failure with proteinuria, hematuria, and anuria developed. Liver function returned to normal 3 days after stopping rifampin. Renal function returned to normal in 3 months. A lymphocyte transformation test to rifampin was negative.—(*From Excerpta Medica*)

Clinical Sciences

Arora, S. K. and Mukhija, R. D. Squamous cell carcinoma in trophic ulcer cases. *Indian J. Lepr.* **59** (1987) 100–102.

Two cases having trophic ulcer over the heel for a long time who later developed squamous cell carcinoma are reported because of its paucity in the literature. Various factors blamed for carcinomatous change are enumerated.—Authors' Abstract

Choudhary, M. S., Singh, N. B., Srivastava, K. and Gupta, H. P. Patterns of immunoglobulins in the serum of leprosy patients. *Indian J. Lepr.* **59** (1987) 178–187.

There have been considerable variations in the levels of immunoglobulins in the serum of leprosy patients which needed elucidation. An attempt has been made in this direction to find out the levels of IgG, IgM, and IgA in the serum samples of 145 cases of various grades of leprosy. The patients were selected in the age group of 11 to 60

years and have received treatment with dapsone for a period ranging from 6 months to 5 years. A constant increase in the levels of immunoglobulins has been noted, and the effect of treatment over variation in the levels has been discussed.—Authors' Abstract

Datz, F. L. Erythema nodosum leprosum reaction of leprosy causing the double stripe sign on bone scan; case report. *Clin. Nucl. Med.* **12** (1987) 212–214.

Leprosy is a disease that is widely distributed in under-developed tropical regions, with cases also occurring in the United States. The bone scan findings in a patient with leprosy who had developed a reactional state of the disease, erythema nodosum leprosum, are presented. His scan showed bilaterally symmetrical double stripe signs involving the distal tibias, similar to those seen in hypertrophic osteoarthropathy.

Physicians should be aware that this reactive phase of leprosy can also produce the double stripe sign.—Author's Abstract

Fornage, B. D. and Nerot, C. Sonographic diagnosis of tuberculoid leprosy. *Ultrasound Med.* **6** (1987) 105–107.

A 27-year-old Guadeloupean man living in France for 2 years received a direct trauma 1 year previously to the postero-lateral aspect of the knee. Weakness and then paralysis of extensor hallucis longus and tibialis anterior muscles progressively developed. At physical examination, a firm fixed mass was palpated posterior and lateral to the head of the fibula, associated with a skin depigmentation. Electromyography demonstrated neuropathy in the distribution of both medial and lateral popliteal nerves. Ultrasound was performed using a 5 MHz linear-array real-time probe and a stand-off pad according to a previously described technique. Longitudinal and transverse scans delineated a globally hypoechoic 5 by 3 by 2 cm relatively well-defined area, fusiform in configuration, abutting the fibula. Two parallel internal linear echoes could be demonstrated on longitudinal sonograms. Surgical exploration revealed a spindle-shaped caseous mass surrounding the markedly enlarged lateral popliteal nerve. The medial popliteal and lateral saphenous nerves were also significantly thickened at their origins. Histologic examination confirmed tuberculoid leprosy with the presence of Hansen's bacilli.—(From the Article)

Fraser, A. G., Croxson, M. S. and Ellis-Pegler, R. B. Hypercalcaemia and elevated 1,25-dihydroxy-vitamin D3 levels in a patient with multibacillary leprosy and a type 1 leprosy reaction. (Letter) *N.Z. Med. J.* **100** (1987) 86.

Hypercalcemia has been described in association with many granulomatous diseases, including sarcoidosis, tuberculosis, fungal infections, berylliosis, and a case of silicone-induced granulomas. Ryzen and Singer recently reported one patient with rheumatoid arthritis and borderline tuberculoid leprosy associated with hypercalcemia which responded to prednisone treatment. The serum 1,25-dihydroxy-vitamin

D3 (calcitriol) concentration was normal, and the mechanism of the hypercalcemia remained speculative. We report a patient with multibacillary leprosy complicated by a type 1 reaction associated with hypercalcemia. The serum calcitriol concentration was raised on two occasions during her convalescence when both the serum calcium and urine calcium excretion were normal.—(From the Letter)

Gelber, R. H., Gooi, H. C., Porterfield, J. S., Lam, S. K. and Rees, R. J. W. Viral challenge in leprosy: viraemia, interferon, and specific antibody production. *Lepr. Rev.* **58** (1987) 155–164.

Following 17-D yellow fever vaccination, viremia and specific neutralizing antibody production were assessed in groups of 12 healthy Malay controls and Malay tuberculoid and lepromatous leprosy patients. Subsequent viremia was found in 10 healthy subjects, 9 tuberculoid patients, and 8 lepromatous patients. Neither the time of appearance, chronicity, nor titer of viremia was different among the three groups. Nine or 10 individuals from each of the three subject groups developed specific neutralizing antibody. Prior to vaccination, the ability of peripheral blood leukocytes to produce interferon *in vitro* after stimulation with Newcastle disease virus was studied. Leukocytes from all the healthy subjects and patients produced significant amounts of interferon. Neither lepromatous nor tuberculoid patients' leukocytes produced levels of interferon different from healthy controls. A tendency was observed for lepromatous patients to produce decreased amounts of interferon *in vitro* as compared to tuberculoid patients.—Authors' Summary

Joseph, M. S. and Charoen, W. S. Problems encountered in treating one leprosy patient in a developing country: a case report. *Lepr. Rev.* **58** (1987) 165–171.

We describe multiple problems encountered in the management of one patient suffering from lepromatous leprosy in a developing country, and these include type 2 lepra reaction, intercurrent infection, shortage of experienced colleagues to consult, laboratory shortcomings, and side effects of

chemotherapy. It is postulated that the development of pustular and acneiform skin lesions in our patient is a new and previously unreported side effect of dapsone.—Authors' Summary

Kumar, B., Koshy, A., Kaur, S., Kaur, I. and Rajwanshi, A. Leprosy, liver and jaundice. *Indian J. Lepr.* **59** (1987) 194–202.

Nine patients of leprosy, 5 BL and 4 LL who developed jaundice during the course of disease, were investigated. Two LL patients developed jaundice during ENL reaction. There was slight hepatomegaly in 5 patients and moderate splenomegaly in 3 only. There were significant alterations in liver enzymes and serum bilirubin in all patients. The abnormalities of the enzyme levels persisted for abnormally long periods even when the serum bilirubin had come down and the patients had become asymptomatic. Blood for HBsAg and anti-HAV IgM was negative in all patients except one in whom HBsAg was positive. Drugs could not be implicated as the cause of jaundice, all patients maintained recovery even after restarting antileprosy drugs. The possibility of nonA, nonB viruses producing hepatitis during the course of disease is brought out. Course of prolonged jaundice in leprosy is compared with other diseases which could result in a similar situation.—Authors' Abstract

Lamfers, E. J. P., Bastiaans, A. H., Mra-vunac, M. and Rampen, F. H. J. Leprosy in the acquired immunodeficiency syndrome. (Letter) *Ann. Intern. Med.* **107** (1987) 111–112.

Among the bacterial infections most suggestive of the acquired immunodeficiency syndrome (AIDS) are those caused by *Mycobacterium avium* complex, *M. kansasii*, and disseminated forms of *M. tuberculosis*. We present the case of an infection with *M. leprae* associated with AIDS, a case that has not previously been reported.

This case report presents three features of interest: a unique combination of the major infectious diseases of leprosy, tuberculosis, and AIDS; a Kaposi sarcoma localized in a hemorrhoid, a feature previously unrecognized; and the unusual presence of leprosy

in an immunocompromised host.—(From the Letter)

Malaviya, G. N., Girdhar, B. K., Hussain, S., Ramu, G., Lavania, R. K. and Desikan, K. V. Scalp lesion in a lepromatous patient—case report. *Indian J. Lepr.* **59** (1987) 103–105.

The occurrence of lesions over the scalp in an Indian lepromatous patient is reported. The patient had central and frontal baldness. The scalp lesion had histological features consistent with lepromatous leprosy.—Authors' Abstract

Mann, S. B. S., Kumar, B., Yande, R., Kaur, S., Kaur, I. and Mehra, Y. N. Eighth nerve evaluation in leprosy. *Indian J. Lepr.* **59** (1987) 20–25.

Twenty-five cases of bacillary-positive leprosy patients and 25 age- and sex-matched controls were investigated for assessment of cochleo-vestibular status. Impaired hearing was complained of by 4 patients. None had tinnitus, dizziness, or vertigo. On testing, 44% of the patients were found to have unilateral or bilateral perceptive deafness. Specialized tests of hearing indicated that the deafness was of the cochlear type. The vestibular functions were not affected. Leprosy seems to selectively involve the cochlea.—Authors' Abstract

Paksoy, N. Evaluation of the clinical and histopathological findings in indeterminate leprosy. *Turkish Arch. Dermatol. Syphilol.* **21** (1987) 93–101. (in Turkish)

According to the present concept, indeterminate leprosy is considered as the early stage of the disease in terms of evolution. Indeterminate leprosy shows diagnostic difficulties both clinically and pathologically. It is also very important to detect the disease in this early stage to prevent further medical and social damage. This study presents clinicopathological analyses of 27 histologically confirmed indeterminate leprosy cases at the Department of Pathology, Schieffelin Leprosy Research and Training Centre, Kari-giri, Vellore, India, in 1985. All of the cases were previously untreated. The results were also aimed to contribute to clinical and histopathological recognition of indeterminate

leprosy cases in Turkey, which is the author's country of origin with 4000 known cases.

Clinical findings: Most the cases (33%) were in the third decade of life. Age ranged from 7 to 58 years (mean age = 27 years); 19 cases were males and 8 were females. The main sign was a single or multiple (up to 3 in number) patches, and this was found in 25 out of 27 cases (92%). Two cases only showed an area of anesthesia without any skin lesion. Macules were hypopigmented in 22 cases (81%) and erythematous in 3 cases (11%). Macule was single and hypopigmented in 12 cases (44%). Number of cases with multiple hypopigmented macules was 8 (29%), and 2 cases showed a maculopapular lesion.

Loss of sensation was present in 22 cases (81%). In 17 cases (65%), duration was 1 year or less. Data on contacts were noted in 16 cases, and 4 cases (25%) showed one or more contacts in their families. In 4 cases (14%), skin lesion was associated with peripheral nerve enlargement. The commonest site of the lesion was the extremities (61%). The thigh was the most frequent specific site (26%). Skin smear was negative in all cases. Lepromin test was applied in only 6 cases, and 4 of these showed slightly positive reactions. Leprosy was clinically considered in 17 of the 27 cases (62%), while it was suspected in 9 cases (33%). One case was clinically thought to have lichen planus.

Main histopathological findings: Atrophy in the epidermis (40%). Lymphohistiocytic infiltration around the capillaries, skin appendages (100%). Peri- and/or intraneural lymphohistiocytic infiltration of the dermal nerves (100%). This finding was also associated with prominent and enlarged appearance of the dermal nerves (51%), Schwann cell proliferation in the nerves (11%), perineural cell proliferation (7%) and perineural thickening (7%). Acid-fast bacilli were detected in various places in 17 cases (63%). Bacilli were detected most commonly in the cell infiltrate in the dermis (26%). Other sites showing bacilli were dermal nerves (14%) and arrector pili muscle (7%). In 6 cases (14%), a focus of epithelioid histiocytes was found within the inflammatory infiltration in the dermis, and these cases were regarded as transforming to the pre-tuberculoid stage of the disease.

Five (18%) of the 27 cases were initially reported to have bacilli. During this study careful screening of the present slides revealed bacilli in 6 more cases (40%). Then fresh slides having 4 sections from each of the remaining 16 "bacilli negative" cases were also prepared. Examination of these slides disclosed bacilli in 6 more cases. This brought the total number of the "bacilli positive" cases to 17 (63%). It shows the importance of careful search of the present slides and preparing some more fresh sections as well.

It is said that indeterminate leprosy should not be diagnosed unless at least an acid-fast bacillus was found in biopsies in nonendemic countries. If the clinical and other above-mentioned histopathological findings are strongly suggestive, indeterminate leprosy could be considered in leprosy-endemic areas even without finding a bacillus.—Author's English Summary

Pavithran, K. Melkersson-Rosenthal syndrome masquerading as tuberculoid leprosy. *Indian J. Lepr.* **59** (1987) 26–29.

A case of Melkersson-Rosenthal syndrome is reported in a middle aged woman. Her skin lesion (cheilitis granulomatosa) resembled tuberculoid leprosy, clinically and histopathologically.—Author's Abstract

Posner, D. I. and Guill, M. A., III. Coexistent leprosy and lupus erythematosus. *Cutis* **39** (1987) 136–138.

We present a patient who, while under therapy with systemic corticosteroids to control lupus erythematosus, noted lesions of leprosy (Hansen's disease) on her elbow and knee. The corticosteroids may have played a role in the emergence of the leprosy. Leprosy should be considered in the differential diagnosis of cutaneous lesions with sensory loss to hasten diagnosis and therapy.—Authors' Abstract

Rajendran, N. Palmo-plantar lesions in paucibacillary leprosy—unusual clinical expressions. *Indian J. Lepr.* **59** (1987) 188–190.

Paucibacillary leprosy of the palmo-plantar skin is regarded as unusual. Three cases of paucibacillary leprosy with such lesions

are discussed and the relevant literature reviewed.—Author's Abstract

Russell, D. Leprosy. *Aust. Fam. Physician* **16** (1987) 113–117.

Leprosy is a chronic infectious disease that mainly affects the skin, superficial cutaneous nerves, eyes, testes, and the upper respiratory tract. The diagnosis of leprosy in recent migrants from South-East Asia stresses the need for an awareness of the disease.—Author's Abstract

Smith, W. C. S. A multicentre evaluation of a questionnaire to assess ability in the diagnosis of leprosy. *Lepr. Rev.* **58** (1987) 141–148.

Case finding in leprosy control programs is dependent on the ability of field staff in

the diagnosis of leprosy. However, assessment of this ability in field circumstances is difficult and time consuming. In this study 20 case histories in the form of a questionnaire are evaluated as a tool to assess ability to interpret the signs and symptoms of leprosy. The study included field workers of different grades and differing experience from six centers in India. The validity of this method is shown by the better performance by the higher grades of staff and the correlation of performance with experience. The use of case histories in this way is a useful educational tool; it can also be used to identify members with major difficulties in the diagnosis of leprosy and to identify particular cases which present more general diagnostic problems.—Author's Summary

Immuno-Pathology

Barros, U., Shetty, V. P. and Antia, N. H. Demonstration of *Mycobacterium leprae* antigen in nerves of tuberculoid leprosy. *Acta Neuropathol. (Berl.)* **73** (1987) 387–392.

Twenty nerve biopsies of tuberculoid leprosy patients, who showed no acid-fast bacilli in their skin smears or in tissue biopsies, were stained for mycobacterial antigens using anti-bacille Calmette-Guérin (BCG) by the peroxidase-antiperoxidase method. Adjacent parts of some of these nerves were examined for the presence of osmiophilic bacilli under the transmission electron microscope. Eight of the 20 nerves were both clinically and histologically uninvolved. All of the 20 involved nerves showed presence of antigen located, mainly intracellularly, in the cytoplasm of epithelioid cells and to a lesser degree in Schwann, endothelial, and plasma cells. A few nerves with caseated nerve abscesses showed clusters of antigen deposits in both the caseous mass as well as the wall of the abscess. In 6 of the 9 nerves processed for electron microscopy, electron-dense bacilli were noted within the cytoplasm of Schwann cells but not within the infiltrating cells. The uninvolved nerves

showed neither antigen deposits nor osmiophilic bacilli despite fine ultrastructural changes. Our observations indicate that a) the specificity of the immune response in paucibacillary nerve lesions is probably against bacterial components. b) There is a differing antigen handling by Schwann cell and the inflammatory epithelioid cell. c) Plasma cells may play a role in presenting antigen. d) *Mycobacterium leprae* may be acting as an adjuvant in causing damage to uninvolved nerves at distal sites.—Authors' Summary

Bharadwaj, V. P., Katoch, K., Ramu, G., Suribabu, C. S. S. and Desikan, K. V. Seroepidemiological studies in subsided cases of multi- and paucibacillary types of leprosy using FLA-ABS test. *Indian J. Lepr.* **59** (1987) 30–35.

The FLA-ABS test was carried out in 145 subsided cases of multibacillary (BL/LL) and 197 subsided cases of paucibacillary (TT/BT/I) types. The period of subsidence varied from 0–5 years or more. It was observed that percentage positivity decreases with the subsidence of the disease. In multibacillary cases, FLA-ABS positivity was 95% (active

cases), 50% (after 1 year of subsidence), and 36% (after 5 years of subsidence); whereas in paucibacillary types FLA-ABS positivity was 80% (active cases), 34% (after 1 year of subsidence), and 19% (after 5 years of subsidence). A significant proportion of multibacillary subsided cases continued to be positive at high titers (1:160 and 1:640) even after 5 years of clinical subsidence. This implies the presence of continuous antigenic stimulus resulting in high antibody titers even after several years of clinical subsidence. On the other hand, percentage positivity at high titers gradually decreased with the increased duration in paucibacillary cases.—Authors' Abstract

Britton, S. Hypothesis: leprosy and the sun. *Ethiop. Med. J.* **24** (1986) 149–153.

It has been known for some time that ultraviolet (UV) light has a strong influence on skin tumors. UV light may induce skin tumors in various species, and these highly antigenic tumors may be transplanted to syngeneic animals that have undergone UV treatment, but not to untreated animals. The immunosuppressive effects of UV light are mainly confined to the skin, as it is known that such light does not penetrate far beyond the skin surface. However, it has been shown in the murine leprosy model that if mice are exposed to UV light over one side of their body surface, then the skin on the opposite side will show decreased resistance to an intradermal injection of *Mycobacterium leprae* bacilli (personal communication, C. C. Shepard, 1985).

Recent experiments in mice indicate that one of the target cells for UV light in the immune system is the antigen-presenting cell (APC), where there is a differential sensitivity of macrophages inducing suppression versus immunity. When adherent cells are presenting antigen favoring suppression, they are insensitive to UV light. At present, it is an unresolved question whether distinct populations of APCs induce suppression versus immunity.

The local immunological response in the skin is dependent upon lymphocytes and APCs, which in the skin are probably multifold, and among which are the Langerhans' cells. The UV sensitivity of these cells in man is not known, but in the murine

delayed hypersensitivity system, they are exquisitely sensitive. If it is assumed that their UV sensitivity in man follows the same pattern as in their murine counterparts, it would mean that exposure to UV light would favor antigen presentation along the suppressive pathway.

The proposition is that the sun may be one important factor in the outcome of *M. leprae* infection, a hypothesis which is testable.—(From the Article)

Britton, W. J., Hellqvist, L., Ivanyi, J. and Basten, A. Immunopurification of radiolabeled antigens of *Mycobacterium leprae* and *Mycobacterium bovis* (bacillus Calmette-Guérin) with monoclonal antibodies. *Scand. J. Immunol.* **26** (1987) 149–159.

Radiolabeled sonicate of *Mycobacterium leprae* when examined by SDS-PAGE and two-dimensional gel electrophoresis (2-DE) contained fewer antigens than the comparable sonicate from *M. bovis* (bacillus Calmette-Guérin) (BCG). A solid-phase immunopurification assay with anti-*M. leprae* monoclonal antibodies (MAB) was used to characterize four of these antigens. Three of the MAB were *M. leprae*-specific and with them antigens with apparent molecular weights (M_r) of 12,000 (12K), 18K, and 35K were isolated. On 2-DE, the heavily labeled 12K antigen was heterogeneous with a range in pI of 4.8–5.2. The 35K antigen, which was identified by a conformational determinant, and the 18K antigen were also acidic proteins with pI of 5.4 and 5.1. The fourth antigen was purified from both *M. leprae* and BCG sonicates and had an M_r of 70K and a pI of 5.1. MAB reacting with the cell wall protein of *M. leprae* resulted in separation of multiple bands ranging in M_r from 12K to 65K, rather than the dominant 65K protein seen in immunoblots. A similar pattern was obtained with MAB that reacted with two cell-wall polysaccharide antigens, and these antibodies may have co-precipitated the radiolabeled cell-wall proteins. Immunoprecipitates of the *M. leprae* sonicate with human lepromatous leprosy sera, when analyzed by 2-DE, were also found to contain the dominant 12K band and the 35K band. Furthermore, half the radiola-

beled BCG antigens were precipitated by the same sera.—Authors' Abstract

de Wit, M. Y. L., van Rens, M. M., van Schooten, W., Hartskeerl, R., Kolk, A. H. J. and Klatser, P. R. Isolation and characterization of the 36K antigen of *Mycobacterium leprae*. *J. Microbiol.* **52** (1986) 463–464.

The isolation of specific antigens derived from *Mycobacterium leprae* might provide the basis of a clinically useful diagnostic test for leprosy and could, in addition, open the way for the design of a *M. leprae* vaccine. Previously we reported the identification of *M. leprae*-specific protein antigens which were recognized by leprosy patients' sera. We have prepared a monoclonal antibody (MAB) which recognized one of these antigens, a 36K protein, and have used this MAB for the isolation of the antigen from *M. leprae*. Previously we had applied this MAB in an ELISA-inhibition test for the serology of leprosy.

Immunochemical and biochemical characterization of the isolated antigen showed that the antigenic determinant recognized by the MAB was insensitive to heat- and trypsin-treatment, but sensitive to pronase-treatment. The MAB was also used as a probe to isolate a recombinant λ gt 11 phage clone, expressing the 36K antigen in *Escherichia coli*. Further analysis of the recombinant DNA products revealed that the MAB reacted with a fusion protein consisting of β -galactosidase and the 4K C-terminal part of the antigen. Therefore, the antigenic determinant must be located within this terminal part. Both the 36 kD antigen from *M. leprae* and from the recombinant phage clone have been shown to stimulate T-helper cell clones from leprosy patients.

Since the antigen has been shown to be involved in both the humoral and cellular immune responses of leprosy patients, continuation of the study at the molecular level should further our understanding of its role during infection with *M. leprae*.—Authors' Abstract

Gonzalez-Abreu, E. and Gonzalez, A. Seroreactivity against the *Mycobacterium leprae* phenolic glycolipid I in mycobacteria-

infected or stimulated groups of individuals. *Lepr. Rev.* **58** (1987) 149–154.

The enzyme-linked immunosorbent assay (ELISA) was applied in a group of sera of lepromatous leprosy patients, tuberculosis patients, BCG-vaccinated children, and blood bank donors using the phenolic glycolipid I, isolated by Hunter and Brennan, for the determination of specific antibodies.

Positive results were found in the group of leprosy patients while the majority of the sera of the other individuals were negative. Slight crossreactivity was encountered in a few individuals. At the same time a study was carried out in healthy persons without a known contact with *Mycobacterium leprae*. These received a lepromin injection (Mitsuda test), and blood samples were taken before and 21, 45, and 90 days afterward. In this case, evidence was shown that the lepromin injection did not influence the results of the test.—Authors' Summary

Li, F., et al. [Serological activity of semi-synthetic polysaccharide antigens of *M. leprae*.] *China Lepr. J.* **1** (1987) 20–23. (in Chinese)

ND-P-BSA and NT-P-BSA are effective antigens for the testing of antibodies specific to *Mycobacterium leprae* in the serum. The serological activity of the NT is higher than that of the ND antigen. The authors consider that the use of the two reagents labeled with the enzyme against both human IgM and IgG in the ELISA is necessary in order to accumulate research materials. Cross-reactivity of the antigens with the serum of the patients having tuberculosis is a problem pending further investigation.—Authors' English Abstract

Mehta, L., Dorvadi, M. H. and Damavandi, N. M. Alkaline phosphatase activity in peripheral nerves and endothelial cell cultures in leprosy. *Indian J. Lepr.* **59** (1987) 171–177.

Alkaline phosphatase activity in leprosy nerves was studied. The activity was mainly in blood vessels and was maximum in healthy nerves. Low levels were seen in crush injury. In leprosy, lower levels were in BT than in LL cases. Endothelial cells (*in vitro*)

released alkaline phosphatase when infected with live bacilli only. No response was observed with heat-killed bacilli.—Authors' Abstract

Montreewasuwat, N., Curtis, J. and Turk, J. L. Interleukin 1 and prostaglandin production by cells of the mononuclear phagocyte system isolated from mycobacterial granuloma. *Cell. Immunol.* **104** (1987) 12–23.

A study has been made of the activity of interleukin 1 (IL-1) and prostaglandins (PGs) in the culture supernatants from unstimulated and lipopolysaccharide (LPS)-stimulated mycobacteria-induced granuloma cells. Both epithelioid cells from bacillus Calmette-Guérin (BCG)-induced granulomas and macrophages from *Mycobacterium leprae*-induced granulomas, separated on a fluorescence-activated cell sorter using monoclonal antibody specific to guinea pig macrophages, spontaneously secreted low levels of IL-1 (assayed by thymocyte mitogenic and fibroblast mitogenic activities) into culture supernatants. However, culture supernatants from LPS-mediated epithelioid cells showed significantly higher IL-1 activity than those from unstimulated cells. In contrast, LPS stimulation of *M. leprae* granuloma macrophages failed to enhance IL-1 production. Nevertheless, IL-1 activity in the culture supernatants from stimulated mycobacterial granuloma cells of both types was much lower than that from LPS-stimulated peritoneal exudate macrophage culture supernatants. There was no detectable amount of prostaglandin E₂ (PGE₂) in the culture supernatants from both unstimulated and LPS-stimulated BCG- and *M. leprae*-induced granuloma cells in comparison to much higher levels of PGE₂ produced by unstimulated (0.28–6.2 ng/ml) or LPS-stimulated (>15 ng/ml) peritoneal exudate macrophages. However, BCG granuloma cells either secreted prostaglandin F(2 α) (PGF(2 α)) spontaneously or produced comparable levels of PGF(2 α) to those from peritoneal exudate macrophages on stimulation, while *M. leprae* granuloma macrophages produced much lower levels of PGF(2 α).—Authors' Abstract

Muthukkaruppan, V., Chakkalath, H. R. and James, M. M. Immunologic unresponsiveness in leprosy is mediated by modulation of E-receptor. *Immunol. Lett.* **15** (1987) 199–204.

By using an indirect immunofluorescence technique with OKT3 and OKT11 monoclonal antibodies, the percentage of CD2 positive cells was found to be reduced in the peripheral blood of bacterial index positive lepromatous leprosy patients; however, in these patients, CD3 positive cells were at the normal level. Further, CD2 positive cells attained the normal proportion in lepromatous patients when mycobacterial load was reduced. Both CD2 and CD3 receptors were expressed at the normal level in tuberculoid leprosy patients. Prior treatment of peripheral blood mononuclear cells from healthy controls with *Mycobacterium leprae* significantly decreased the percentage of CD2 but not CD3 positive cells. Such a modulation of CD2 on T cells also resulted in blocking the lymphoproliferative response induced by mitogen and antigen. These results suggest that there is a strong correlation between CD2 modulation and immunologic unresponsiveness in leprosy.—Authors' Summary

Pinto, M. R. M., Eriyagama, N. B. and Pemajayantha, V. Studies of reactivity of some Sri Lankan population groups to antigens of *Mycobacterium leprae*. I. Reactivity to lepromin A. *Lepr. Rev.* **58** (1987) 105–118.

This paper reports a survey of lepromin reactivity in adult population groups in areas at three different elevations (geographical localities) in central Sri Lanka, using a lepromin A with a bacillary content of 3 or 4 \times 10⁷ bacilli/ml. The patterns of reactivity observed with both Fernandez and Mitsuda reactions were clearly bimodal and similar in all areas. The distributions of reactions were divisible into "non-reactor" ("negative") and "reactor" ("positive") components. For both Fernandez and Mitsuda reactivity, the demarcation between non-reactor and reactor components seemed to be best made at a reaction size of 3 mm. The mode of reactors of the Fernandez re-

action was at 3–6 mm, and of the Mitsuda reaction at 5–8 mm. Both types of reactivity showed no change with increase of age. Fernandez reactivity showed no evidence of any change with sex, race, BCG vaccination status, or geographical area. Mitsuda reactivity did not seem to be affected by race or geographical area, but there seemed to be possible changes with sex and BCG vaccination status. Even so, there seems to be a trend for higher reaction sizes in males, and the BCG vaccinated, with both types of reactivity.—Authors' Summary

Pinto, M. R. M., Eriyagama, N. B., Pe-majayantha, V. and Fish, D. G. Immunological effects of lepromin testing in Sri Lankan population groups. I. Effect of repeated lepromin testing. *Lepr. Rev.* **58** (1987) 119–128.

It has been reported that lepromin testing in human subjects induces sensitization, and that with repeated testing the incidence of positive Mitsuda reactions increases. On repeated testing in two Sri Lankan population groups with Mitsuda reactions of 6 mm or less, we found that a second lepromin test at 28 days seemed to induce tolerance with reduction in reaction size or even zero reactions. This tolerance phenomenon was seen markedly with Mitsuda reactivity and less so with Fernandez reactivity. There was evidence also that while tolerance seemed to be occurring with the second test, a third test at 56 days seemed to reinduce and elicit re-sensitization, though weakly, with both types of reactivity. Evidence is also produced that reactors and non-reactors with both Fernandez and Mitsuda reactivity behave differently on repeated lepromin testing, suggesting that immunologically they are different population groups.—Authors' Summary

Porichha, D. and Bhatia, V. N. Epithelioid and polygonal cells in histoid leprosy. *Indian J. Lepr.* **59** (1987) 191–193.

Salient histological features of 36 histoid leprosy are presented. Usual features, like whorls and interlacing bundles of spindle cells, histoid habitus of the bacilli, were no-

ticed in all the cases. Pseudocapsule was present in 6 cases. Circumscribed islets of epithelioid cells were seen in the deeper part of the granuloma in 5 cases. Acid-fast bacilli were absent or rare in these cells. Polygonal, foamy macrophages were found in 12 cases. The presence of epithelioid and polygonal cells in histoid leprosy is rare and its significance needs to be explored.—Authors' Abstract

Schmutzhard, E., Fuchs, D., Hausen, A., Reibnegger, G. and Wachter, H. Evaluation of urinary neopterin—a marker of cell-mediated immune response—in patients with leprosy. In: *Biochemical and Clinical Aspects of Pteridines, Vol. 3. Cancer, Immunology, Metabolic Diseases*. Pfeleiderer, W., Wachter, H. and Curtius, C. H., eds. Berlin: Walter de Gruyter & Co., 1984, pp. 483–490.

Neopterin, a pyrazino-(2,3-d)-pyrimidine compound, is specifically released from macrophages induced by either supernatants from allogeneically activated T lymphocytes or gamma interferon. *In vivo*, urinary neopterin excretion has been demonstrated to be an excellent parameter for clinical activity of diseases which involve activation of cellular immunity. In almost 80% of leprosy patients urinary neopterin excretion is elevated, when compared with healthy controls. No difference was seen between the tuberculoid and the lepromatous type of leprosy. This observation seems to be indicative that neopterin excretion reflects a systemic activation of cellular immunity in leprosy patients rather than differences in the distribution of T-cell phenotypes which are known to be localized at the site of the lesions within the skin and the nerves.—(From the Article)

Sehgal, V. N., Srivastava, G. and Beohar, P. C. Histoid leprosy—a histopathological reappraisal. *Acta Leprol. (Genève)* **5** (1987) 125–131.

Twenty-three clinically diagnosed histoid leprosy patients were subjected to histopathological and histochemical investigations. The histopathological features were composed of a well-formed lesion sur-

rounded by a pseudocapsule. In addition, a free, mildly eosinophilic, uninvolved sub-epidermal zone, and variously arranged spindle-shaped histiocytes were noted in the lesion. Polygonal and foamy histiocytes were also seen in a few sections. The lesion also had a large number of solid-staining, acid-fast lepra bacilli. The latter might have stimulated a peculiar histiocytic tissue response almost identical to that seen in histiocytoma(cutis) but for the absence of hemosidrin and lipids.—Authors' Abstract

Sibley, L. D., Franzblau, S. G. and Krahenbuhl, J. L. Intracellular fate of *Mycobacterium leprae* in normal and activated mouse macrophages. *Infect. Immun.* **55** (1987) 680–695.

Mycobacterium leprae replicates within mononuclear phagocytes, reaching enormous numbers in the macrophage-rich granulomas of lepromatous leprosy. To examine the capability of macrophages to digest *M. leprae*, we studied the intracellular fate of *M. leprae* organisms in normal and activated mouse macrophages by using the electron-dense, secondary lysosome tracer Thoria Sol. Intracellular *M. leprae* organisms, surrounded by a characteristic electron-transparent zone, were contained within phagosomal vacuoles of macrophages cultured *in vitro* for 1 to 6 days. In normal macrophages, a majority of phagosomes containing freshly isolated live *M. leprae* cells resisted fusion with Thoria Sol-labeled lysosomes. The extent of fusion was not significantly affected by pretreatment of *M. leprae* with human patient serum high in specific immunoglobulin G and M antibodies. In contrast, a majority of phagosomes containing gamma-irradiated *M. leprae* cells underwent lysosome fusion in normal macrophages. In addition, increased phagolysosome fusion was observed with live *M. leprae*-containing phagosomes in macrophages activated with gamma-interferon. Increased fusion was associated with an increase in the number of fragmented and damaged bacilli, suggesting that increased digestion followed fusion. This study indicates that activated macrophages may have an increased capacity for clearance of nor-

mally resistant *M. leprae*.—Authors' Abstract

Vaccines against leprosy. *Lancet* **1** (1987) 1183–1189.

Three leprosy vaccine trials—in Malawi, Venezuela, and India—have now been started. The hope that a safe and effective vaccine against leprosy could be developed has been fueled for several years by the observation that individuals who do not respond to *Mycobacterium leprae* can be induced to do so by immunization with various agents, including the leprosy bacillus itself.

Recent approaches to vaccine production have involved two strategies—use of *M. leprae* itself or of immunologically related, nonpathogenic organisms other than BCG. Of the three new trials, two (Venezuela and Malawi) have used *M. leprae* (given, in both cases, in combination with BCG). This vaccine was developed as part of the World Health Organisation Special Programme for Research on the Immunology of Leprosy (the "IMMLEP vaccine"); *M. leprae* is grown in armadillos, purified from the infected tissue, and killed by irradiation. The third trial (in Maharashtra, India) uses a mycobacterium which, unlike *M. leprae*, can be grown *in vitro*; this organism was originally cultured at the Indian Cancer Research Campaign Laboratories and is known as the "ICRC bacillus."

The purpose of the trials in Malawi and Venezuela is to investigate the protective effect of the *M. leprae*-BCG combination. In Malawi, a population of approximately 120,000 is being studied in the northern district of Karonga. The trial is jointly supported by IMMLEP and the British Leprosy Relief Association (LEPRA), who have been carrying out a leprosy evaluation project in the area since 1979. The effect of vaccination with *M. leprae* plus BCG is being compared with that of BCG alone on the incidence of disease; preliminary results should be available within 5 years. The Venezuelan trial also uses the *M. leprae*-BCG combination, and 20,000 contacts of leprosy patients have been vaccinated. The ICRC vaccine trial in Maharashtra is concentrating on healthy household contacts of leprosy

patients; it is hoped that 100,000 contacts will be vaccinated and followed up for 7 years.—(From the Article)

Vergheese, S., Curtis, J. and Turk, J. L. Epithelioid cell granuloma induction in the guinea pig by haptened *Mycobacterium leprae*. *Cell. Immunol.* **107** (1987) 307–316.

Fluorescein isothiocyanate (FITC)-conjugated *Mycobacterium leprae* (FITC-*M. leprae*) was injected intradermally into the ears of guinea pigs and granuloma formation in the draining postauricular lymph nodes was studied. At 2 weeks, there was an increase in weights and infiltration of the draining lymph nodes in half of the animals injected with FITC-*M. leprae*. At 5 weeks, there was a significant increase in the weights and infiltration of these draining lymph nodes in the guinea pigs injected with haptened *M. leprae* compared with those injected with unconjugated *M. leprae*. At 5 weeks, there was also a significant increase in delayed-type hypersensitivity responses to 25 µg purified protein derivative. Histologically, epithelioid cell granulomas were seen in these lymph nodes as early as 2 weeks when FITC-*M. leprae* was used as the source of antigen. Enhancement in the immuno-

genicity of *M. leprae* by conjugation with FITC has been postulated.—Authors' Abstract

Wiker, H. G., Harboe, M., Nagai, S., Patarroyo, M. E., Ramirez, C. and Cruz, N. MPB59, a widely crossreacting protein of *Mycobacterium bovis* BCG. *Int. Arch. Allergy Appl. Immunol.* **81** (1986) 307–314.

The MPB59 protein of *Mycobacterium bovis* BCG was purified to homogeneity from culture fluid of BCG substrain Tokyo, and characterized by biochemical and immunological techniques. The molecular weight was 28,000, determined by SDS-polyacrylamide gel electrophoresis, and the pI value was 5.3. The N-terminal amino acid sequence was determined for 32 steps, and showed no significant homology with MPB64, MPB70, or MPB80. By crossed immunoelectrophoresis, MPB59 was found to belong to the BCG antigen 85 complex and identified as corresponding to the 85B component of this complex. The protein crossreacted extensively with other species of mycobacteria, and induced a marked humoral immune response in armadillos and monkeys during development of systemic mycobacterial infection after inoculation with *M. leprae*.—Authors' Abstract

Microbiology

Bhagria, A. and Mahadevan, P. R. A rapid method for viability and drug sensitivity of *Mycobacterium leprae* cultured in macrophages and using fluorescein diacetate. *Indian J. Lepr.* **59** (1987) 9–19.

The ability of viable *Mycobacterium leprae* to hydrolyze fluorescein diacetate (FDA) and retain fluorescein inside the bacteria was used to identify viable *M. leprae* inside cultured *in vitro* macrophages. The subjective microscopic count of the FDA test was demonstrated as a useful routine test by confirming the results obtained therein with a quantitative and nonsubjective measurement of fluorescence in a spectrofluorimeter. Using this method loss of viability of *M. leprae* in the presence of dapsone and rifampin was demonstrated. Such an assay

was well correlated with another *in vitro* assay, the Fc receptor test and also the *in vivo* mouse foot test. The drug resistance of clinical isolates of *M. leprae* demonstrated by mouse foot pad was also correlated with the FDA test system. Thus, we have reported a reliable, consistent, and rapid *in vitro* test system for determining viability and drug sensitivity of *M. leprae*.—Authors' Abstract

Bharadwaj, V. P., Katoch, V. M., Sharma, V. D., Kannan, K. B., Datta, A. K. and Shivannavar, C. T. Metabolic studies on mycobacteria. IV. Assay of isocitrate lyase and malate synthase activity in *M. leprae*. *Indian J. Lepr.* **59** (1987) 158–162.

Cell-free extracts from *Mycobacterium tuberculosis* H37Rv, *M. smegmatis*, arma-

dillo-derived *M. leprae* and normal armadillo liver homogenates were assayed for the presence of isocitrate lyase and malate synthase activity. It was observed that significant amounts of isocitrate lyase and malate synthase activity were present in *M. tuberculosis* H37Rv, *M. smegmatis* and armadillo-derived *M. leprae*. No such activity was demonstrable in cell-free extracts of normal armadillo liver. It is concluded that *M. leprae* like other mycobacteria has the capability to metabolize via glyoxylate bypass of TCA cycle. These findings may be relevant for understanding the energy metabolism of *M. leprae* under stress conditions and possibly the "persister" stage.—Authors' Abstract

Fujiwara, T., Aspinall, G. O., Hunter, S. W. and Brennan, P. J. Chemical synthesis of the trisaccharide unit of the species-specific phenolic glycolipid from *Mycobacterium leprae*. *Carbohydr. Res.* **163** (1987) 41–52.

O-(3,6-Di-*O*-methyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-*O*-(2,3-di-*O*-methyl- α -L-rhamnopyranosyl)-(1 \rightarrow 2)-3-*O*-methyl-L-rhamnopyranose, the haptenic trisaccharide of the *Mycobacterium leprae*-specific phenolic glycolipid I (PGL-I) antigen, and related trisaccharides were synthesized by allylation of *O*-2 of benzyl 4-*O*-benzyl- α -L-rhamnopyranoside using phase-transfer catalysis, methylation of the product, deallylation, and coupling to *O*-(2,4-di-*O*-acetyl-3,6-di-*O*-methyl- β -D-glucopyranosyl)-(1 \rightarrow 4)-2,3-di-*O*-methyl-L-rhamnopyranosyl bromide or related disaccharides. Anomeric mixtures of the trisaccharide derivatives were separated by preparative thin-layer chromatography, deacetylated, and hydrogenolyzed, to give the pure trisaccharides. It had already been demonstrated that only those trisaccharides containing an intact, terminal 3,6-di-*O*-methyl- β -D-glucopyranosyl unit are effective in inhibiting the specific binding between PGL-I and anti-PGL-I immunoglobulin M antibodies in human lepromatous leprosy sera.—Authors' Abstract

Ibegbu, C., Mandock, O., Kale, V. and Nalkar, R. G. Studies on the antigenic specificity of *Mycobacterium leprae*. I. Isoelectric and chromatofocusing separa-

tion. *Zentralbl. Bakteriolog. Mikrobiol. Hyg. [A]* **264** (1987) 67–77.

Cell sonicates of *Mycobacterium leprae* and other mycobacteria were subjected to isoelectric focusing and chromatofocusing to evaluate their protein antigens and to determine if the patterns were significantly different. Isoelectric focusing showed that the proteins of all mycobacteria focused within the pH range of 3.5 to 5.5, except those of *M. leprae* which extended beyond 5.5 to 6.5. These studies have indicated for the first time that the protein antigens of mycobacteria are acidic in nature. Comparison between the proteins of untreated and autoclaved *M. leprae* showed distinct differences between the two preparations, in respect of loss of some antigens in the autoclaved *M. leprae* sonicate. This indicates that the bands that were not visible in the autoclaved *M. leprae* were those of heat-labile proteins. It is possible, however, that the absent bands could have been of a low order of intensity and hence were not discernible. On the other hand, the proteins could have coagulated due to the heat treatment, thus causing confirmational changes or ionic interactions with membrane components, due to their acidic nature. It is possible that the proteins in the autoclaved *M. leprae* are the ones that possess immunogenic properties since the protective ability of heat-killed *M. leprae* has already been established. Chromatofocusing studies have confirmed the isoelectric focusing data in respect to the number of antigens and their respective protein content, besides permitting the availability of the various fractions for further biological characterization.—Authors' Abstract

Kato, L. Factors affecting growth of the metabolically competent but microbe-dependent *Mycobacterium leprae* (?); indications for *in vitro* cultivation in a multifactorial medium. *Ann. Immunol. Hung.* **26** (1986) 939–958.

A multifactorial liquid medium (MFM) was used to grow *Mycobacterium leprae* (?). The medium was prepared by inoculating *M. phlei* into iron-free Sauton medium with 1% Tween 80 added. After 20 days of incubation at 37°C, the culture was autoclaved and filtered on paper. In 1 L filtrate Na

thioglycolate $(\text{NH}_4)_2\text{SO}_4$ 2 g, thiocetic acid 0.1 g, ferric ammonium citrate 0.05 g, and MgSO_4 0.1 g were dissolved, distributed in screw-cap tubes, and autoclaved. Optimal growth of *M. leprae* (?) was obtained at pH 5.8, 34°C and at reduced O_2 tension. Primary cultures from host-grown (armadillo) *M. leprae* cells were not obtained on the 3% agar slants of the MFM. Host-grown *M. leprae* cells were incubated for 20 days at 34°C as deep cultures in liquid MFM, and the sediment was transferred to the surface of MFM agar slants. Positive cultures were obtained in 60 to 90 days as a matt, somewhat yellowish confluent growth on the surface of the agar slants. Subcultures were obtained from both the liquid and 3% agar MFM. Cultures were strongly acid-fast and did not grow on Löwenstein or 7H10 media. The presence of both exochelins and mycobactin, as well as compounds containing the SH group, was essential for growth of *M. leprae* (?) in the MFM. The designation *M. leprae* (?) is proposed before characterization of the cultures is completed. — Author's Abstract

Katoch, V. M., Katoch, K., Bharadwaj, V. P., Datta, A. K., Sharma, V. D., Shivannavar, C. T. and Kannan, K. B. Metabolic studies on mycobacteria. V. A preliminary report on the ATP synthesis by mycobacteria including *M. leprae* by using different substrates. *Indian J. Lepr.* **59** (1987) 163–170.

By deletion and addition of various substrates in Sauton's and Dubos media, an experimental system has been standardized in which the role of various nutrients in the energy synthesis of mycobacteria can be determined. By using this system with cultivable mycobacteria it was observed that glycerol and asparagine are the important ingredients for ATP synthesis by mycobacteria. Glucose further enhanced the ATP synthesis and growth of these mycobacteria. In the media containing asparagine or glycerol, there was marginal increase in the ATP in the *Mycobacterium leprae* suspensions initially, but this was not sustained and there

was no progressive increase in biomass or multiplication. When *M. leprae* were incubated in the media from which both of these substrates were deleted, there was progressive decline in ATP levels right from the beginning. From these preliminary results, it appears that asparagine and glycerol may be useful as substrates for the ATP synthesis by *M. leprae* and need to be investigated further. In-depth studies are necessary to find out the factor(s) which results in the inability of *M. leprae* to utilize these and other substrates in a sustained manner for its multiplication and growth in artificial media. — Authors' Abstract

Katoch, V. M., Sharma, V. D., Kannan, K. B., Datta, A. K., Shivannavar, C. T. and Bharadwaj, V. P. Metabolic studies on mycobacteria. III. Demonstration of key enzymes of TCA cycles in *M. leprae*. *Indian J. Lepr.* **59** (1987) 152–157.

Cell-free extracts of armadillo-derived *Mycobacterium leprae*, *M. phlei*, *M. smegmatis* and normal armadillo liver were analyzed for the two key enzymes of TCA cycle. Aconitase activity was assayed in the presence of inhibitor fluorocitrate and it was observed that cell-free extracts from cultivable mycobacteria as well as armadillo-derived *M. leprae* had this enzyme activity and 66–82% of this activity was inhibited by 0.1 mM fluorocitrate; 74% of *M. leprae*-derived enzyme activity was inhibited by fluorocitrate in contrast with armadillo-derived enzyme which was only 29% inhibited by fluorocitrate. PAGE separation of cell-free extracts and staining for isocitrate dehydrogenase (ICD) activity showed that an additional band of ICD activity was demonstrable in the cell-free extracts of armadillo-derived *M. leprae* and this was NADP dependent. The mobility (ef) of this band of activity was in the same range as ICD from cultivable mycobacteria and much lower than ICD from normal armadillo liver. From this study and from the previously reported work, it is concluded that as in other mycobacteria the TCA cycle is operative in *M. leprae*. — Authors' Abstract

Experimental Infections

Baskin, G. B., Gormus, B. J., Martin, L. N., Wolf, R. H., Blanchard, J. L., Malaty, R., Walsh, G. P., Meyers, W. M. and Binford, C. H. Experimental leprosy in African green monkeys (*Cercopithecus aethiops*): a model for polyneuritic leprosy. *Am. J. Trop. Med. Hyg.* **37** (1987) 385-391.

Three African green monkeys (*Cercopithecus aethiops*) were inoculated intravenously and intracutaneously with *Mycobacterium leprae* derived from a naturally infected mangabey monkey. All developed cutaneous lesions at inoculation sites. One developed disseminated cutaneous lesions, while the cutaneous lesions in the other two regressed and eventually disappeared. The animals were examined at necropsy 5 years after inoculation. All three had active leprosy infection in peripheral nerves with extensive inflammation and fibrosis. The disease histologically resembled borderline-lepromatous leprosy. These findings add a new dimension to animal models of leprosy.—Authors' Abstract

Gelber, R. H. Activity of minocycline in *Mycobacterium leprae*-infected mice. *J. Infect. Dis.* **156** (1987) 236-239.

These studies demonstrate that minocycline is unique among the tetracyclines because it is active against *Mycobacterium leprae* infection in mice. The plasma MIC for *M. leprae* was exceedingly low, <0.2 µg/ml. This is much lower than the MIC previously found to be effective *in vitro* against minocycline-susceptible mycobacteria. After treatment, which resulted in higher plasma concentrations (0.6-0.9 µg/ml), greater activity against *M. leprae* was observed. These levels are well within the clinically achievable range: after a customary adult dose of 100 mg of minocycline administered twice daily, plasma levels remain at 2-4 µg/ml. Furthermore, the activity of minocycline seems to be consistently bactericidal and, when compared with results of published studies using similar methods (except for rifampin and possibly ethionamide), more bactericidal for *M. leprae* infection in mice

than are other agents currently utilized to treat leprosy.—(From the Article)

Job, C. K. Transmission of leprosy. *Indian J. Lepr.* **59** (1987) 1-8.

Experimental studies have shown that the two common routes of entry of *Mycobacterium leprae* are the skin and the nasal mucosa, and that the organisms multiply at the site of entry and produce a local lesion before the disease is disseminated. Ordinarily, the first demonstrable lesions in humans are in the skin. There are many patients in whom there is convincing evidence to prove that entry of *M. leprae* occurred at the site of the primary skin lesion. But search for primary lesions in the nasal mucosa has not been very successful.

It has been postulated that in humans when *M. leprae* enter through the nasal mucosa lepromatous disease results and that skin entry of *M. leprae* causes tuberculoid disease. In experimental leprosy, both skin and nasal entry of *M. leprae* have resulted in lepromatous or borderline disease. Tuberculoid disease as seen in humans has never been produced in animal models.

With further experimental studies using highly susceptible armadillos and the newly discovered monkey models and with careful observations in epidemiological studies this question can be finally resolved.—(From the Editorial)

Natori, T., Saito, N., Matsuoka, M., Suzuki, S., Mizuno, K., Matsuno, Y., Niijama, T., Fujii, H. and Aizawa, M. The phagocytic activity of macrophages correlates with the RT1.B-restricted immune response to *Mycobacterium leprae-murium* in the rat. *Transplan. Proc.* **19** (1987) 3190-3193.

To clarify the functional role of the major histocompatibility complex (MHC) gene products in infectious diseases, we investigated an experimental leprosy in terms of its strain difference in susceptibility, immune responsiveness, and macrophage function in the rat. Several laboratories, including ours, reported an important role of the MHC in resistance of *Mycobacterium*

lepraemurium (MLM) infection in mice. We have previously demonstrated that the unresponsiveness to soluble MLM in CBA/J mice is due to the generation of suppressor T cells and that Ia-restricted, MLM-induced, T-cell proliferative response is highly correlated with the high phagocytic activity of macrophages to live MLM bacilli. In this report we describe the comparative findings in MLM infection in the rat.

When the two parameters, intensity of lymphocyte proliferative responses and that of phagocytic activity of macrophages, are considered, they appear to be closely associated, i.e., a close correlation between the two was observed in the nine different strains of rats.

RT1.B-restricted MLM-induced lymphocyte proliferative responses are highly associated with the phagocytic activity of macrophages to MLM bacilli. The phagocytic activity is also correlated with the resistance to MLM infection, which resulted

in a reduced growth of MLM bacilli.—(From the Article)

Stallknecht, D. E., Truman, R. W., Hugh-Jones, M. E. and Job, C. K. Surveillance for naturally acquired leprosy in a nine-banded armadillo population. *J. Wildl. Dis.* **23** (1987) 308–310.

Samples from 77 nine-banded armadillos (*Dasypus novemcinctus*) inhabiting a 16.7 km portion of the East Atchafalaya River Levee, Pointe Coupee Parish, Louisiana, U.S.A., were serologically tested and/or histopathologically examined for evidence of naturally acquired leprosy. Five of 67 (7.5%) armadillos tested seropositive with ELISA test for IgM class antibodies to the phenolic glycolipid-I antigen of *Mycobacterium leprae*. One of 74 (1.3%) was histopathologically positive as determined by the presence of acid-fast bacteria in nerves.—Authors' Abstract

Epidemiology and Prevention

Bian, T., et al. [Effect of leprosy control in Baoying County, Jiangsu Province.] *China Lepr. J.* **1** (1987) 16–18. (in Chinese)

A plan for the elimination of leprosy in Baoying County by the year 1996 was made. In this county, the annual prevalence and incidence of leprosy have decreased from a peak of 2.85% and 22.06/100,000 to 0.14% and 1.01/100,000, respectively, in the last 30 years. Among new patients, the rate of children has decreased from 26.77% to 2.59%, and no child patient was found in the 1980s. The age of patients at onset has moved from 24 years to 34–44. The period of the disease at beginning of the treatment has shortened from 64.3 to 15 months. The number of villages where patients are found declined from 97.9% to 21.3%. The results indicate that the comprehensive control measures for leprosy are effective. The rate of progressive decrease of prevalence and incidence over the years suggested that the incidence and prevalence will be 0.2/100,000 and 0.01%, respectively, by the year 1996. The authors put forward some con-

crete suggestions for controlling leprosy in the future.—Authors' English Abstract

Fu, Z., et al. [Leprosy control in the city of Fuzhou.] *China Lepr. J.* **1** (1987) 14–16. (in Chinese)

The authors present the history of leprosy endemicity and the measures and effects of leprosy control in the city of Fuzhou, Fujian Province, People's Republic of China. A total of 1739 patients were found until the end of the year 1984. Of these, 1564 cases have been cured and there are 64 active patients with a prevalence of 0.05/million now in the city.—Authors' English Abstract

Gundersen, S. G. Leprosy and tuberculosis in the Blue Nile Valley of western Ethiopia. *Lepr. Rev.* **58** (1987) 129–140.

Data are presented on the prevalence of leprosy and tuberculosis among different ethnic groups living under different environmental conditions in the Mendi district of the Blue Nile Valley, Western Ethiopia.

The data are based on a clinical survey of 1323 persons (main study), representing Highland and Midland Oromos as well as Midland and Lowland Nilotics, and on records from the local leprosy and tuberculosis programs (additional study). It is concluded that cases of leprosy are rarely found in the highlands, whereas prevalences of 53/1000 and 92/1000, respectively, were found in two of the Lowland Nilotic villages. On the other hand, tuberculosis is 2–4 times more frequent among the Highland and Midland Oromo population (10–18/1000) than among the Midland and Lowland Nilotics (3–7/1000). In addition to the genetic difference between the Oromo and Nilotic populations, the higher temperatures, lower humidity, and black soil observed at the lower altitudes might be of importance for the prevalence of mycobacterial disease. There is some evidence of an ongoing leprosy epidemic among the previously isolated Lowland Nilotics, and indications of a tuberculosis epidemic starting after their increased contact with the tuberculosis-infested Highland Oromos.

This paper is the first in a series that reports on the prevalences of several public health problems in this area, the Blue Nile Public Health Survey (BNPHS). Hence, some general information is provided on the concerned population, the geography, and the organization of the public health services in the Mendi district.—Author's Summary

Jiangqu Hospital. [Clinical analysis of 969 leprosy patients in Jiangqu Hospital of Tibet.] *China J. Lepr.* **1** (1987) 12–13. (in Chinese)

Jiang Qu Leprosarium of the Xizang (Tibet) Autonomous Region has accepted and treated 969 leprosy patients in the last 20 years, of whom lepromatous cases accounted for 65.9% and borderline for 19.6%. Their ages mostly were from 20 to 30 years and 488 cases have been cured. The duration of the disease at the patient's admission was longer than 2 years and the discovery of patients in the earlier stage of the disease is very difficult because leprosy control workers are very few.—Authors' English Abstract

Lechat, M. F., Misson, C. B., Vanderveken, M., Vellut, C. M. and Declercq, E. E. A computer simulation of the effect of multidrug therapy on the incidence of leprosy. *Ann. Soc. Belg. Med. Trop.* **67** (1987) 59–65.

Epidemiometric models are used for studying the dynamics of a disease in a population. In order to help decision makers, the simulated impact of multidrug therapy (MDT) on leprosy incidence is compared with standard dapsone monotherapy. Simulations over 20 years show that the reduction obtained with MDT is quite considerable, whether given to all patients or exclusively either to multibacillary or to paucibacillary patients. Neither high relapse rates after MDT nor earlier detection significantly influence the difference observed between MDT and monotherapy.—Authors' Summary

Louis, F. J., Louis, J.-P., Schill, H. and Parc, F. [Last offensive of leprosy in the South Pacific Ocean: the outbreak of Rapa (1922–1950).] *Bull. Soc. Pathol. Exot. Filiales* **80** (1987) 306–319. (in French)

Observations of leprosy outbreaks are rare and almost always occur in isolated areas of the world. An epidemic of leprosy occurred in Rapa, a small island in French Polynesia, from 1922 to 1950. The different parameters of the epidemic were studied by retrospective methods and fieldwork. After peaks of 11 per 1000 incidence rate and 68 per 1000 prevalence rate, the outbreak decreased. Today leprosy is endemosporeadic on the island.—(From Authors' English Summary)

Miranda, R. F. and Fernández, R. M. G. [The history and epidemiology of leprosy in the province of Almeria.] *Fontilles Rev. Leprol.* **16** (1987) 45–50. (in Spanish)

The historical evolution of leprosy in the province of Almeria, Spain, that was introduced by the Phoenicians in the cities of Barea and Abdera in the 7th to 5th centuries B.C. is studied. The leprosy endemic and its distribution in public health districts is also analyzed as a step toward integration of the reforms of the Primary Health Service in Andalucia.—Authors' English Abstract.

Pan, Y., et al. [Analysis of all cases of newly detected leprosy in the years 1983–1985 in Shandong Province.] *China Lepr. J.* **1** (1987) 18–20. (in Chinese)

Nine hundred-eleven new leprosy patients discovered during 1983–1985 were analyzed. The results show that a general survey is not suitable because it is too expensive, and few patients can be found by this method in an area with low endemicity villages. Where there have been leprosy patients, the area should be surveyed, since the incidence of leprosy there is higher. Children less than 10 years of age do not have to be examined since only extremely rarely are patients to be found among them. The authors suggest that to find new patients in early stages, measures such as skin disease clinics, clue survey, examination of the family members of leprosy patients, giving rewards to the reporter of a patient, and teaching diagnostic understanding to the doctors in general hospitals are very important for diagnosing early leprosy.—Authors' English Abstract

Thomas, D. A., Mines, J. S., Thomas, D. C., Mack, T. M. and Rea, T. H. Armadillo exposure among Mexican-born patients with lepromatous leprosy. *J. Infect. Dis.* **156** (1987) 990–992.

A leprosy-like illness identical to that produced in captive armadillos by inoculating them with *Mycobacterium leprae* from humans has been observed in wild armadillos (*Dasybus novemcinctus*) in Louisiana, in Texas, and most recently in Mexico. This finding has focused interest on the relationship between leprosy in the armadillo and that in humans. An initial direct examination of the relationship in Louisiana produced no evidence of a link. Case reports from Texas of leprosy occurring in humans after exposure to armadillos, however, emphasize the continued pertinence of the question. We conducted a small case-control study to assess armadillo exposure in Mexico among individuals who have subsequently migrated to Los Angeles and received care for clinical leprosy. A history of direct contact with armadillos was strongly associated with leprosy in both sexes, es-

pecially in men; past indirect exposure to armadillos was more commonly described by patients of either sex than by controls. The degree of association was similar whether first exposure occurred before or after age 10. Only 9 patients and 4 controls were aware of any past contact with humans who had leprosy; exclusion of these 13 subjects did not affect the results.

Patients and controls also differed on a number of potentially confounding factors. These included the region of origin (a minority of subjects, but more patients than controls, came from the northern states of Mexico) and the size of the residential community of origin in Mexico (cases more commonly came from smaller communities). Men with leprosy also reported excessive direct exposure to iguanas and deer.

Associations with past armadillo exposure were adjusted for age, size of town, region of origin, and history of exposure to iguanas and deer by using an unconditional multiple logistic analysis that treated exposure both as a binary and as a continuous variable. The potential sources of confounding could not explain our positive findings. In men, the adjusted odds ratio (an accurate estimate of relative risk that is, under these circumstances, presumed to be quite accurate for direct exposure to armadillos was 6.5 and for indirect exposure, 2.7. In women, the adjusted odds ratio for direct exposure was 4.1 and for indirect exposure, 3.5.—(From the Article)

Xiao, J., et al. [General situation of leprosy control in the area of the Qiang nationality in Sichuan Province.] *China Lepr. J.* **1** (1987) 13–14. (in Chinese)

The authors present the general situation of leprosy control among the Qiang nationality of Sichuan Province, People's Republic of China. There accumulatively 302 leprosy cases were registered, of whom 241 cases have been cured. There are 31 active patients presently and both the prevalence and incidence of leprosy have been decreasing. The distribution of the patients was in the form of groups or individual points and the ages of most of the patients were over 50 years.—Authors' English Abstract

Rehabilitation

Boucher, P. and Hirzel, C. [Remote results of decompression of posterior tibial nerve to prevent plantar ulcers and their recurrences.] *Acta Leprol. (Genève)* **5** (1987) 39–45. (in French)

Among patients having had the decompression of the posterior tibial nerve (PTN) from 1979 to 1982 we had been able to find again 42 and the results of their operations assessed after a length of time varying from 3 to 6 years.

Twenty-three patients (40 PTN) have been operated and simultaneously treated by an anti-inflammatory treatment for a tibial neuritis without a plantar ulcer. When they were checked up the severe or slight cases showed only little change. Among the incomplete ones 70% of total recovery of the plantar sensitivity is noted and no plantar ulcer is found. Three patients out of the 10 cases of complete insensitiveness having not improved showed a plantar ulcer.

Nineteen patients (23 PTN) underwent a surgical decompression for plantar ulcers. Healing was achieved in all cases except 3. When they were checked up only 4 cases have not recurred, all the others, about 80%, have seen the ulcers coming back at least 2 or 3 recurrences during 5.5 years. The rate of the recurrences seems to be connected with the stage of deformation of the foot, the lack of wearing special sandals, and the fact of not taking into account the advice of health advisers.

Our impression is that the PTN decompression has obviously shown more effectiveness at the stage of incomplete anesthesia of the sole to prevent the coming out of a first plantar ulcer than a stage of a settled ulcer because in this case it does not seem alone to avoid a recurrence.—Authors' English Summary

Breger, D. Correlating Semmes-Weinstein monofilament mappings with sensory nerve conduction parameters in Hansen's disease patients: an update. *J. Hand Ther.* **1** (1987) 33–37.

Until recently there has been little documentation to show whether there is a cor-

relation between Semmes-Weinstein monofilament mappings and sensory nerve conduction tests. The data presented in this paper include a group of 73 patients diagnosed with Hansen's disease, with 142 median and ulnar nerves studied. The patients measured had both tests completed within 5 days of each other. In 72% of the cases, both tests were in agreement, having results either within normal limits or both testing abnormal or absent. These tests cannot be expected to directly correlate; they can only be compared. Although there are essential differences in these two tests, when used together they present a clearer picture of the patient's sensory status than either test used alone.—Author's Abstract

Fritschi, E. P. Field detection of early neuritis in leprosy. *Lepr. Rev.* **58** (1987) 173–177.

Five field tests for detecting early damage to peripheral mixed nerve trunks in leprosy are described. The regular monthly use of these tests will assist in reducing deformity. The whole set can be administered in less than ten seconds.—Author's Summary

Gerochi, L. N. Knowledge, beliefs and attitudes on leprosy in Iloilo City proper, Philippines. *Southeast Asian J. Trop. Med. Pub. Health* **17** (1986) 433–436.

This study attempts to find out the prevailing knowledge and beliefs in Iloilo City, The Philippines, regarding leprosy, its causation, mode of transmission, and care. Attitudes of people toward leprosy patients and their offspring are also looked into. Qualitative data are obtained from seven key persons in the locality who were interviewed in depth using the unstructured interview guide. Quantitative data are furnished by 100 household heads who were randomly chosen and by 70 senior student nurses enrolled in the six colleges of nursing in the same city using an interview schedule and questionnaire, respectively.

Results reveal that 90% of the household heads and 45% of the students have inadequate knowledge on leprosy. None of the

household heads and only two students have a highly adequate knowledge pertaining to this disease.

Leprosy is believed by both the household heads and students to be brought about by germs as well as supernatural causes. A great majority of the household heads (94%) and students (79%) attribute the cause of leprosy to germs. Blood predilection has been cited by the next highest proportion of both groups of respondents. The third highest proportion of the households' heads attribute the cause of the illness to God's will, while among the students, the cause of this disease is believed to be spontaneous.

The majority of household heads (94%) and students (95%) believe that skin-to-skin contact is the most possible mode of transmitting the disease.—Author's Summary

Guo, C., et al. [Treatment of neuralgia and prevention of deformity with endoneurolysis.] *China J. Lepr.* **1** (1987) 8–10. (in Chinese)

The authors report the results of 38 cases of nerve pain which were treated by intraneurolysis. There was good effect on pain relief and the prevention of deformity. Two cases of failure were due to post-operative infection. Intraneurolysis on ulnar and peroneal nerves is discussed.—Authors' English Abstract

Jacobs, J. M., Shetty, V. P. and Antia, N. H. Myelin changes in leprosy neuropathy. *Acta Neuropathol. (Berl.)* **74** (1987) 75–80.

Myelin changes were observed in fibers of nerves from cases of leprosy. The myelin had a "loosened" appearance caused by increased and irregular separation of the intraperiod line. "Loosening" might affect all, or only some, of the lamellae forming a myelin sheath. There was a patchy distribution of fibers with abnormal myelin, and they were seen only in nerves showing other marked pathological changes including the presence of edema. The appearances are suggestive of intramyelinic edema which may be related to the presence of endoneurial edema.—Authors' Summary

Jacobs, J. M., Shetty, V. P. and Antia, N. H. Teased fibre studies in leprosy neuropathy. *J. Neurolog. Sci.* **79** (1987) 301–313.

Nerve biopsies were taken from four cases of leprosy, which included borderline tuberculoid, borderline, and lepromatous types. They were examined in 1- μ m resin sections and in teased preparations. The most common finding in teased fibers from each leprosy type was paranodal demyelination affecting successive internodes. In transverse sections some fibers showed changes associated with axonal atrophy. Together, these findings suggest that demyelination in some cases may be secondary to axonal changes. In addition, there was evidence of focal areas of demyelination affecting whole internodes of many fibers at the same level across the nerve and which was possibly caused by local factors.—Authors' Summary

Jiang, Z., et al. [Effect of treating 255 cases of leprosy plantar ulcer.] *China Lepr. J.* **1** (1987) 6–8. (in Chinese)

A total of 255 cases of plantar ulcer were treated by the following methods: excision of the metatarsal heads, free skin graft, dorsal island flap of foot, free muscle and skin flap of toes, soaking in *Ilex pubescens* Hook et Arn, dressing with sugar, local immobilization, dressing with zinc plate or amnion. The results were satisfactory, but recurrences were common. The authors emphasize that the prevention of plantar ulcer is most important in leprosy control programs.—Authors' English Abstract

Zhang, G., et al. [Surgical correction of deformed thumbs following paralysis of nervus ulnaris.] *China Lepr. J.* **1** (1987) 4–6. (in Chinese)

Several surgical methods for the correction of thumb deformity due to ulnar nerve paralysis are described. The indications and results of the operations (excellent = 31, good = 12, poor = 3) as well as the arthrodesis of the thumb and other problems are discussed.—Authors' English Abstract

Zheng, T., et al. [A report on correcting lagophthalmos in 26 leprosy patients with

transplantation of a fascicle of the temporalis.] *China Lepr. J.* **1** (1987) 2–3. (in Chinese)

The results of temporalis bundle transfer with a fascia lata graft to correct lagophthalmos are reported. The technique of opera-

tion, the assessment of results, and the experiences are described. The authors are of the opinion that this method may be a routine operation for lagophthalmos.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Bahr, G. M., Chugh, T. D., Behbehani, K., Shaaban, M. A., Abdul-Aty, M., Al Shimali, B., Siddiqui, Z., Gabriel, M., Rook, G. A. W. and Stanford, J. L. Unexpected findings amongst the skin test responses to mycobacteria of BCG vaccinated Kuwaiti school children. *Tubercle* **68** (1987) 105–112.

A multiple skin-test survey was carried out in Kuwait on 1200 school children aged 8–11 years, and on 1228 children aged 12–16 years. With only 15 exceptions, all of these children had received vaccination with Japanese BCG just before they started school, 5 years and 9 years earlier, respectively. Tuberculin positivity was almost 90% in both groups, with a mean response size of 8.7 mm. This was associated with remarkably high responsiveness to many of the other mycobacterial species investigated. Since this high reactivity was also to *Mycobacterium ulcerans*, a species most unlikely to be present in Kuwait, it is proposed that this might be due to responsiveness to group ii antigen which is present in all slow-growing species.

Only *M. flavescens* and *M. rhodesiae* among the fast-growing species were absent as sensitizing organisms. After correction for the supposed reactivity to group ii antigen, *M. avium B*, *M. gordonae*, *M. ulcerans* and *M. xenopi* among the slow-growing species also appeared to be absent from the Kuwait environment. The species most commonly encountered were *M. leprae*, *M. chitae*, *M. neoaurum*, *M. diernhoferi*, and *M. vaccae* in this order. This was a remarkable finding for a country assumed to be poor in contact with environmental species, and known to have a very low prevalence

of leprosy. As previously reported from Iran, but not confirmed in other places, there was a 95% correlation between responsiveness to leprosin A and Vaccin. Among the slow-growing species, *M. avium A*, *M. intracellulare*, and *M. kansasii* appear to be frequent sensitizing agents, in common with many other places.—Authors' Summary

Britton, W. J., Hellqvist, L., Basten, A. and Inglis, A. S. Immunoreactivity of a 70 kD protein purified from *Mycobacterium bovis* bacillus Calmette-Guérin by monoclonal antibody affinity chromatography. *J. Exp. Med.* **164** (1986) 695–708.

The protein antigens from *Mycobacterium bovis* (BCG), *M. tuberculosis*, and *M. leprae* share a number of common determinants. We have used a murine mAb (L7) recognizing such a determinant on a protein of M(r) 70,000 to purify this antigen from *M. bovis* sonicate by affinity chromatography. Enrichment of the protein in column eluates was confirmed by immunoblotting and in antigen inhibition assays. After radiolabeling with ¹²⁵I, the protein could be immunoprecipitated with human lepromatous leprosy sera. Stimulation of peripheral blood mononuclear cells from BCG-vaccinated and naturally Mantoux-positive individuals induced proliferation and IFN- γ secretion, while intradermal injection of purified antigen into the same subjects resulted in a delayed-type hypersensitivity reaction. Thus, the 70,000 molecule carried epitopes capable of reacting with B cells, and eliciting a potentially protective T-cell response. The first 15 N-terminal residues were sequenced using a gas-phase sequenator.—Authors' Abstract

Casal, M., Gutierrez, J., Gonzalez, J. and Ruiz, P. *In vitro* susceptibility of *Mycobacterium tuberculosis* to a new macrolide antibiotic: RU-28965. *Tubercle* **68** (1987) 141–143.

The *in vitro* susceptibility of *Mycobacterium tuberculosis* to a new macrolide antibiotic, RU-28965, alone and in combination with rifampin or isoniazid, was studied by the agar dilution method. At a concentration of 4 mg/l or lower of RU-28965, 90% of the strains were inhibited. A synergistic effect with both rifampin and with isoniazid was demonstrated.—Authors' Summary

Cocito, C. and Vanlinden, F. Preparation and properties of antigen 60 from *Mycobacterium bovis* BCG. *Clin. Exp. Med.* **66** (1986) 262–272.

Antigen 60 (A60) is the main thermostable immunogen of both "old tuberculin" (OT) and purified protein derivative (PPD), known reagents for cutaneous tests in tuberculosis. It is recognized by bidimensional immunoelectrophoresis with anti-BCG antiserum, where it appears as the less mobile component. A60 was prepared from the cytoplasm of *Mycobacterium bovis* BCG, and purified by exclusion gel chromatography and lectin affinity chromatography. Labeled A60 was obtained by radioiodination and used for a radioimmunoassay. Composition of A60 was explored by use of organic solvents, chemicals and enzymes. It contained two fractions of free and bound lipids, as well as protein and polysaccharide moieties. After removal of both free and bound lipid fractions, the core still retained the ability to form immunoprecipitinogen lines with anti-BCG antiserum. The lipopolysaccharide and lipo-protein moieties of A60, as well as the free lipid fraction, were also complexed by antibodies. It is concluded that A60 is a lipopolysaccharide-protein complex of 10^6 to 10^7 daltons, which is a major immunogenic component of mycobacterial cytoplasm. The detailed structure of this antigen, its immunological properties, and its use for an ELISA type immunoassay for tuberculosis are described in two other publications.—Authors' Abstract

Cox, R. A. and Katoch, V. M. Evidence for genetic divergence in ribosomal RNA genes in mycobacteria. *FEBS Lett.* **195** (1986) 194–198.

DNA was isolated from *Mycobacterium phlei* and from *M. smegmatis*. Each DNA sample was restricted with endonucleases, the fragments were separated by agarose gel electrophoresis and transferred to nitrocellulose film. Fragments of DNA containing rRNA sequences were identified by means of ^{125}I -labeled rRNA of *M. phlei* or of *M. smegmatis*. The distributions of restriction endonuclease sites within the rRNA gene(s) and flanking sequences were found to be characteristic for each of the two species. Hybridizations with heterologous probes indicate that although *M. phlei* rRNA and *M. smegmatis* rRNA share regions of sequence homology, they are probably not identical in primary structure. The results suggest that the rRNA genes might prove to be useful taxonomic markers for mycobacteria.—(From *Excerpta Medica*)

Crawford, J. T. and Bates, J. H. Analysis of plasmids in *Mycobacterium avium-intracellulare* isolates from persons with acquired immunodeficiency syndrome. *Am. Rev. Respir. Dis.* **134** (1986) 659–661.

Twenty-six strains of the *Mycobacterium avium* complex isolated from persons with AIDS were examined for plasmid content. The strains were of serotypes 4, 8, 4/6, and 4/8. Plasmid content was determined by agarose gel electrophoresis. All 26 strains carried small plasmids (9 to 15 Mdal), with 11 strains carrying 1 small plasmid and 15 carrying 2. Ten strains also carried large plasmids (>100 Mdal), and 1 strain carried a 60 Mdal plasmid. We have reported molecular cloning of plasmid pLR7, a small plasmid derived from a serotype 4 strain. Cloned segments of pLR7 were used as hybridization probes to detect homologous plasmids in the AIDS-associated strains. Each of the 26 strains carried a plasmid closely related to pLR7. Considerable heterogeneity of size and restriction sites was observed. The finding of plasmids in all strains raises the possibility that they may play a role in virulence. The plasmids will serve as a useful marker for epidemiology

and might be useful as probes for detecting *M. avium-intracellulare* in clinical material.—(From *Excerpta Medica*)

Daffé, M., Lacave, C., Lanéelle, M.-A. and Lanéelle, G. Structure of the major triglycosyl phenol-phthiocerol of *Mycobacterium tuberculosis* (strain Canetti). *Eur. J. Biochem.* **167** (1987) 155–160.

Phenol-phthiocerol glycolipids have been found previously in *Mycobacterium leprae*, *M. kansasii*, *M. bovis*, and *M. marinum*, but not in *M. tuberculosis*. A search for such glycolipids in this latter species showed that the Canetti strains of *M. tuberculosis* synthesize a major triglycosyl phenol-phthiocerol, accompanied by minor amounts of other glycolipids with a similar aglycone moiety. The triglycoside moiety has the following structure: 2, 3, 4-tri-*O*-methyl L-fucopyranosyl ($\alpha 1 \rightarrow 3$) L-rhamnopyranosyl ($\alpha 1 \rightarrow 3$) 2-*O*-methyl L-rhamnopyranosyl ($\alpha 1$ -). The aglycone moiety consists in phenol-phthiocerol (two homologs). Its two secondary alcohol functions are esterified by mycocerosic acids (homologs with 26–32 carbon atoms and with 2–4 methyl branches). The proposed structure differs on several points from the *M. leprae* glycolipids, but presents some analogy with the major glycolipid of *M. kansasii*. A minor monoglycosyl phenol-phthiocerol was also studied. Its overall structure is very similar to that of *M. bovis*, with 2-*O*-methyl rhamnose as sugar moiety.—Authors' Abstract

Dhariwal, K. R., Liav, A., Vatter, A. E., et al. Haptenic oligosaccharides in antigenic variants of mycobacterial C-mycosides antagonize lipid receptor activity for mycobacteriophage D4 by masking a methylated rhamnose. *J. Bacteriol.* **168** (1986) 283–293.

The simple apolar C-mycosides, i.e., structurally well-defined hydrophobic glycopeptidolipids of several mycobacterial species were earlier shown to behave as receptors for adsorption of mycobacteriophage D4. This phage is usually virulent for *Mycobacterium smegmatis*. More complex, polar C-mycosides with additional carbohydrate substituents attached solely to the

deoxytalose have recently been described. They are the highly specific serotyping antigens discovered by W. B. Schaefer—lipids which characterize members of the *M. avium-intracellulare-scrofulaceum* (MAIS) complex. Both kinds are depicted in the structure: fatty acyl-D-Phe-D-aThr[-O-diacyl-6-deoxytalose-O-X]-D-Ala-L-alanine-methylated rhamnose, where X equals H (for simple, apolar C-mycosides) and X equals small oligosaccharides (for antigenic forms; more complex, polar C-mycosides). The present investigations showed that the purified polar antigenic lipids exhibit considerably less adsorptive activity for D4 than do the apolar C-mycosides. Thus, the haptenic oligosaccharides are believed to shield the site in the molecule that the phage recognizes, and the blocking is reinforced by the specific antibodies that the antigens elicit. Although the MAIS serovars usually also produce the phage-reactive apolar C-mycosides, they are not permissive hosts for D4, nor do whole cells adsorb the phage. We suggest that in these species the apolar forms are probably covered at the cell surface by the antigenic lipids. Therefore, these antigenic mycosides may play a putative role in virulence of the MAIS members by protecting these mycobacteria from their own potential pathogen. The results of chemical transformations at specific sites of the mycoside core coupled with studies of simple synthetic lipid glycosides indicated that the principal phage receptor activity resides in the terminal methylated rhamnose. It is this sugar which is evidently masked by the (seemingly remote) haptenic oligosaccharides.—(From *Excerpta Medica*)

Dickinson, J. M. and Mitchison, D. A. *In vitro* activity of new rifamycins against rifampicin-resistant *M. tuberculosis* and MAIS-complex mycobacteria. *Tubercle* **68** (1987) 177–182.

Comparisons were made of the *in vitro* activity of rifampin, and the rifamycin derivatives, rifapentine, rifabutin, CGP 29861, CGP 7040 and CGP 27557, against rifampin-sensitive and rifampin-resistant strains of *Mycobacterium tuberculosis* and against the *M. avium/intracellulare/scrofulaceum* (MAIS) complex. The new rifamycins had

MICs four to eight times lower than those of rifampin against sensitive *M. tuberculosis* strains. Of the 35 rifampin-resistant strains of *M. tuberculosis*, 31% were sensitive to rifabutin but only 3–11% to the other rifamycins. The proportions of the MAIS strains found to be sensitive were 35% for rifampin, 50–60% for CGP 27557, rifapentine and rifabutin, and 85–92% for CGP 29861 and CGP 7040.—Authors' Summary

Dickinson, J. M. and Mitchison, D. A. *In vitro* properties of rifapentine (MDL473) relevant to its use in intermittent chemotherapy of tuberculosis. *Tubercle* **68** (1987) 113–118.

In a comparison of *in vitro* properties of rifapentine (RIF) and rifampin (RMP), the minimal inhibitory concentration of RIF against *Mycobacterium tuberculosis* in Tween-albumin liquid medium was usually 0.02 µg/ml, 2–3 times lower than for RMP; the bactericidal activity against a log phase culture was slightly less than that of RMP and the recovery after pulsed exposures to 1 µg/ml of RIF or RMP lasting 6, 24, and 96 hr was identical for the two rifamycins. These findings are used to interpret published data from the chronic experimental murine tuberculosis model and support the view that in the mouse the efficacy of RIF in widely spaced intermittent chemotherapy is the result of its long half-life.—Authors' Summary

Dickinson, J. M. and Mitchison, D. A. *In vitro* observations on the suitability of new rifamycins for the intermittent chemotherapy of tuberculosis. *Tubercle* **68** (1987) 183–193.

The bactericidal activity of six new rifamycin derivatives—rifabutin (RBU), FCE 22250 (F22), rifapentine (RPE), CGP 29861 (C29), CGP 7040 (C70) and CGP 27557 (C27) and rifampin (RMP)—have been measured against log phase and, as a better test of sterilizing activity, against stationary phase cultures of *Mycobacterium tuberculosis* H37Rv. The order of activity of 1.0 and 0.2 mg/l rifamycin against log phase cultures was RMP > RPE & C27 > RBU & C29 > C70. The order of activity of 1.0 and 0.4 mg/l, adjusted for stability of the

rifamycin, against stationary phase cultures was F22 & RMP > RBU > RPE > C27 & C29 > C70. Viable counts were done during and after pulsed exposures of 6, 24 or 96 hr to C29 and RMP. The curves were similar though C29 was less bactericidal and the lag period before recovery was 1–2 days longer. F22, having high bactericidal activity against stationary organisms and a long half-life, was considered likely to be the most effective sterilizing drug.—Authors' Summary

Donets, O. T., Pislaryuk, L. D., Ilitsky, I. G. and Shcherbina, L. B. [Informative value of liquid-crystalline thermography of reaction to Mantoux test in estimation of tuberculosis activity in children and adolescents.] *Probl. Tuberk.* **5** (1987) 35–37. (in Russian)

Tuberculin sensitivity was studied in children and adolescents with various forms of respiratory tuberculosis of various activity and in children and adolescents with aggravated nonspecific relapsing bronchitis associated with tuberculous infection. Reaction to the Mantoux test with 2 TU was estimated with liquid-crystalline thermoin-dicator films. The difference in the temperatures in the site of tuberculin application and in the symmetrical area of the other forearm in patients with active tuberculosis of the respiratory organs was statistically higher than that in patients with inactive tuberculosis and in the children and adolescents with aggravated nonspecific relapsing bronchitis associated with tuberculous infection.—Authors' English Abstract

Ellis, M. E. and Tayoub, F. Adrenal function in tuberculosis. *Br. J. Dis. Chest* **80** (1986) 7–12.

Plasma cortisol levels were found to be reduced in 55% of 41 Zulus admitted to hospital in Durban, South Africa, with acute pulmonary tuberculosis. Adrenal corticosteroid function improved with therapy, but 30% still showed an impaired response to ACTH. The improvement was less marked in patients receiving rifampin in their drug regimen. This may be a result of the induction of hepatic enzymes by rifampin. The authors suggest that severe Addisonian crisis may be a cause of sudden death in tu-

berculosis patients. [This postulate warrants further investigation.]—M. Hooper (*From Trop. Dis. Bull.*)

Gilburd, B. S. [Determination of IgG antibodies to tubercle bacilli antigens in patients with pulmonary tuberculosis by enzyme immunoassay.] *Probl. Tuberk.* **6** (1987) 61–63. (in Russian)

The content of specific IgG antibodies to PPD and the antigen from the *Mycobacterium bovis* BCG cytoplasm (AGC) was determined with enzyme immunoassay (EIA) in serum of 30 patients with active tuberculosis of the lungs and 20 healthy volunteers. The antigen was isolated by immunosorbent affinity chromatography. The results of EIA showed that the mean values of the content of IgG antibodies to PPD and AGC in the sera were significantly higher as compared to those in the healthy volunteers: 1.2 ± 0.068 and 0.5 ± 0.05 ($p = 0.001$) for PPD and 1.29 ± 0.056 and 0.47 ± 0.07 ($p < 0.001$) for AGC, respectively. Sensitivity and specificity of EIA with the use of PPD amounted to 76.6% and 85%, respectively. The respective figures for EIA with the use of AGC were 90% and 95%.—Author's English Abstract

Gudz, E. A. and Prilutsky, A. S. [Immunological reactivity of patients with various levels of tuberculosis activity.] *Probl. Tuberk.* **3** (1987) 44–47. (in Russian)

The immunological status was studied in 65 patients including 15 patients with infiltrative tuberculosis, 30 patients with active tuberculosis and 20 patients with inactive tuberculomas. Dependence of the immunobiological status on the form and phase of the process was shown. Infiltrative tuberculosis was characterized by a low nonspecific and specific cellular immunity, hyperproduction of IgG and IgA, lower cortisone levels. Activation of tuberculomas was accompanied by increasing nonspecific and specific cellular immunity, hyperproduction of IgG, IgA and IgM, lower cortisone levels. In stabilization of the process, a marked decrease in the level of IgM and an increase in the levels of IgG and IgA were observed.—Authors' English Abstract

Lamb, J. R. and Young, D. B. A novel approach in the identification of T-cell epitopes in *Mycobacterium tuberculosis* using human T-lymphocyte clones. *Immunology* **60** (1987) 1–5.

Current approaches to the analysis of antigens involved in the cellular immune response to mycobacterial infection rely on the initial identification and isolation of molecular components using monoclonal antibodies. In order to overcome the constraints of this approach, we have utilized a procedure involving T-cell recognition of antigens fractionated by polyacrylamide gel electrophoresis (SDS-PAGE) and added to proliferation assays after blotting onto nitrocellulose membranes. Analysis of human T-cell responses to *Mycobacterium tuberculosis* and *M. bovis* BCG by this procedure revealed distinctive patterns of reactivity to different molecular weight components indicative of the selective recognition of immunodominant and species-specific determinants. Human T-cell clones were subsequently derived, and SDS-PAGE immunoblotting was used to identify the antigen recognized by each clone. Three epitopes defined by individual T-cell clones were identified on separate polypeptides with molecular weights 16,000–18,000 (clone P53), 18,000–20,000 (clone P57) and 52,000–55,000 (clone P35). This study demonstrates the potential application of T-cell cloning in conjunction with SDS-PAGE immunoblotting for the dissection and analysis of the cellular immune response to pathogenic agents during human infection.—Authors' Abstract

Mohan, C., Kumar, A. and Agarwal, S. C. Serodiagnosis of pulmonary & extrapulmonary tuberculosis by enzyme linked immunosorbent assay of IgG antibodies using BCG "pressate" antigen. *Indian J. Med. Res.* **85** (1987) 367–373.

Specific IgG class antibody titers against BCG "pressate" antigen were determined in preselected patients with active pulmonary tuberculosis and extrapulmonary tuberculosis and control subjects by enzyme-linked immunosorbent assay (ELISA). Significantly high titers were observed in active pulmonary tuberculosis. Children

having tubercular meningitis and primary tuberculosis (primary complex) showed significantly raised antibody titers. However, their overall mean ELISA titers were lower than those of patients with active pulmonary tuberculosis. Significantly high titers were not detected in localized pulmonary tubercular infections.—Authors' Abstract

Moore, M. B., Newton, C. and Kaufman, H. E. Chronic keratitis caused by *Mycobacterium gordonae*. *Am. J. Ophthalmol.* **102** (1986) 516–521.

We treated a patient with chronic keratitis caused by *Mycobacterium gordonae*, a slow-growing, atypical mycobacterium not previously reported as a cause of corneal infection. The patient was a 34-year-old man who was hit in the eye with some vegetable matter while gardening. Initially, the patient was treated for a presumptive diagnosis of herpes simplex keratitis. Because of progression of the keratitis, a lamellar corneal biopsy was performed 3½ years later and the definitive diagnosis was made. Subsequently, a penetrating keratoplasty was performed and the patient's condition then remained stable. The diagnosis of atypical mycobacterium should be considered in a patient with an indolent corneal ulcer. Lamellar corneal biopsy may disclose the pathogen when the infection is deep, chronic, or partially treated.—Authors' Abstract

Onwubalili, J. K., Edwards, A. J. and Palmer, L. T4 lymphopenia in human tuberculosis. *Tubercle* **68** (1987) 195–200.

Lymphocyte subpopulations *in vitro* in 13 patients with bacteriologically proven tuberculosis and 12 matched controls by immunofluorescence using monoclonal antibodies have been studied. Active tuberculosis was associated with significant reductions in absolute numbers of total T (Leu 4 or 1+), T4 (Leu 3a+), and B (Leu 12+) lymphocytes, but there were no significant differences in total T8 (Leu 2a+) counts. In two patients, T4 lymphopenia was sufficiently profound to cause reversal of T4:T8 ratio (less than 1.2). These changes were not related to the radiological extent of the disease or size of the Mantoux reaction. Normal ranges for the different classes of lym-

phocytes were readily restored by chemotherapy.—Authors' Summary

Parthasarathy, R., Sarma, G. R., Janardhanam, B., Ramachandran, P., Santha, T., Sivasubramanian, S., Somasundaram, P. R. and Tripathy, S. P. Hepatic toxicity in South Indian patients during treatment of tuberculosis with short-course regimens containing isoniazid, rifampicin and pyrazinamide. *Tubercle* **67** (1986) 99–108.

Results are presented of the incidence of hepatitis, nearly always with jaundice, among 1686 patients in clinical trials of the treatment of spinal tuberculosis, of tuberculous meningitis and of pulmonary tuberculosis with short-course regimens containing rifampin, isoniazid, streptomycin and pyrazinamide. The incidence was high in patients treated with daily regimens of isoniazid and rifampin: 16–39% in children with tuberculous meningitis, 10% in patients with spinal tuberculosis (nonsurgical cases), and 2–8% in those with pulmonary tuberculosis. Hepatitis in those receiving rifampin occurred more often in slow than in rapid acetylators of isoniazid, the proportions among those whose acetylator phenotype had been determined being 11% of 317 slow acetylators and 1% of 244 rapid acetylators. In children with tuberculous meningitis, the risk of hepatitis with isoniazid 20 mg/kg (39%) was higher than that with 12 mg/kg (16%), and appreciably lower in patients given rifampin twice weekly (5%) rather than daily (21%). There was no indication that pyrazinamide contributed to the hepatic toxicity.—Authors' Summary

Shi, S., et al. [Thalidomide in the treatment of 50 cases (60 treatments) of lupus erythematosus.] *Chin. J. Clin. Dermatol.* **16** (1987) 72–74. (in Chinese)

The authors had used thalidomide in the past 2 years to treat 50 cases (60 treatments) of lupus erythematosus with an effective rate of 95% and a cure rate of 65%. The results were most satisfactory in SCLE with DDLE next. The dosage recommended was 200 mg/d and tapered down to the maintenance dose when the lesions subsided. It was ineffective for the accompanying joint pain.

In 62.5% of cases the titer of the ANA test reversed to negative or doubtful. The side reactions which occurred in order of frequency were: somnolence, dizziness, oral dryness, fatigue, morbidiform rashes, etc. Contraceptive measures should be adopted in the course of treatment to avoid the possibility of fetal anomalies. In this series, 6 cases relapsed 3–12 months after discontinuation of the drug. The dosage, time of administration, and prevention of side reactions are discussed.—(From Authors' English Abstract)

Shoemaker, S. A., Fisher, J. H., Jones, W. D., Jr. and Scoggin, C. H. Restriction fragment analysis of chromosomal DNA defines different strains of *Mycobacterium tuberculosis*. *Am. Rev. Respir. Dis.* **134** (1986) 210–213.

As an initial step in gaining a better understanding of the important clinical properties that vary between strains of mycobacteria, we attempted to find molecular markers that would define different strains of *Mycobacterium tuberculosis*. We used restriction fragment analysis with the endonuclease Mbol and hybridization with total *M. tuberculosis* DNA to examine DNA differences between 15 strains of *M. tuberculosis*. We were able to identify different strains using this method. In order to assess the sensitivity of this method in identifying different strains, we compared it with phage typing. The two methods appear to be similar in sensitivity and also to be complementary. There were two examples where restriction fragment analysis did not separate strains with different phage types. In addition, there were two examples where phage typing did not separate strains with different restriction patterns. Finally, there were two epidemiologically unrelated strains with the same restriction pattern and the same phage type. This method of restriction fragment analysis of chromosomal DNA is potentially useful for epidemiologic studies of tuberculosis. Additionally, by analyzing the genome of *M. tuberculosis*, molecular markers may well be defined that will be useful in discovering the pathogenesis of the clinical properties of *M. tuberculosis*, which previously have been poorly understood.—(From *Excerpta Medica*)

Singapore Tuberculosis Service/British Medical Research Council. Long-term follow-up of a clinical trial of six-month and four-month regimens of chemotherapy in the treatment of pulmonary tuberculosis. *Am. Rev. Respir. Dis.* **133** (1986) 779–783.

The authors report on further long-term (5–8 years) follow-up of a clinical trial in Singapore of 6-month and 4-month chemotherapy for pulmonary tuberculosis. The earlier studies are confirmed, namely, that a 6-month regimen (2 months daily with streptomycin, isoniazid, rifampin, and pyrazinamide followed by daily isoniazid and rifampin) gave excellent results even with bacilli resistant to either isoniazid or streptomycin or both.—M. Hooper (From *Trop. Dis. Bull.*)

Stanford, J. L., Sheikh, N., Bogle, G., Baker, C., Series, H. and Mayo, P. Protective effect of BCG in Ahmednagar, India. *Tubercle* **68** (1987) 169–176.

As part of a series of investigations to determine the effect of sensitization by environmental mycobacteria on the efficacy of BCG vaccination in India, this study was carried out in Ahmednagar in Maharashtra. A preliminary skin-test survey showed that the rate of sensitization with age was much lower than in Agra, the site of a previous study, and BCG vaccination scars were associated with considerable enhancement in sensitization to tuberculin and other reagents. It was possible to set up prospective BCG vaccination studies in pre-school and primary and secondary school children. Follow ups with skin tests were carried out 1 and 2 years later. By the second year, results were obtained almost identical with those 10 years after BCG administration in the U.K. On this basis it is proposed that the vaccine is likely to provide a considerable level of protection in Ahmednagar. The results of this study also resemble those obtained in the very youngest age group studied in Agra. The marked differences between Indian towns strongly suggest the influence of exposure to mycobacteria in the environment.—Authors' Summary

Steiner, P., Rao, M., Mitchell, M. and Steiner, M. Primary drug-resistant tu-

berculosis in children. Emergence of primary drug-resistant strains of *M. tuberculosis* to rifampin. *Am. Rev. Respir. Dis.* **134** (1986) 446–448.

A prospective study of primary drug-resistant strains of *Mycobacterium tuberculosis* among children was begun at the Kings County Hospital Medical Center of Brooklyn, New York, U.S.A., in 1961 and reported at five 4-year periods through 1980. The present report extends our observations of primary drug-resistant tuberculosis in children through 1984. The salient finding in the present report was the increase in primary drug resistance to rifampin, 3 of 19 strains resistant in the last period of study (1981 to 1984) as compared with 1 of 96 strains isolated in the previous three periods of study (1969 to 1980). This increase was significant ($p < 0.02$) even though the number of strains isolated was small. There were continued low-resistance rates to ethambutol and para-aminosalicylic acid, and stable resistance rates for isoniazid and streptomycin.—(From *Excerpta Medica*)

Stokes, R. W., Orme, I. M. and Collins, F. M. Role of mononuclear phagocytes in expression of resistance and susceptibility to *Mycobacterium avium* infections in mice. *Infect. Immun.* **54** (1986) 811–819.

The growth of *Mycobacterium avium* 702 in the spleens and livers of four inbred strains of mice varied such that the mice could be separated into naturally susceptible (BALB/c and C57BL/6) and naturally resistant (A/Tru and DBA/2) strains. This phenomenon was independent of the size of the infecting inoculum of bacteria in that both low (10^4)- and high (10^7)-dose inocula of *M. avium* grew progressively in susceptible strains and were eliminated from the target organs of resistant strains. Resistance and susceptibility were also demonstrated in *in vitro* preparations of macrophages from these strains of mice. Over a 7-day period, replication of *M. avium* in susceptible mouse macrophages was far greater than that in resistant macrophages. Evidence was obtained to suggest that toxic oxygen metabolites were not responsible for this difference. Although no difference was found in the rate of clearance of *M. avium* from the

blood of susceptible or resistant mice, resident macrophages from susceptible mice ingested more *M. avium in vitro* than did resident macrophages from resistant animals. Growth of *M. avium* in spleens of susceptible mice induced a large influx of phagocytes; whereas this was not observed in resistant mice. In contrast to this it was found that, after injection of a variety of inflammatory agents, influx of leukocytes into the peritoneal cavity could not be used to distinguish susceptible and resistant strains of mice.—Authors' Abstract

Tarasov, A. S., Yakovleva, T. A., Donchenko, A. S., Lisichenko, G. M. and Sandler, B. B. [Role of various mycobacterial species in epidemiology of tuberculosis.] *Probl. Tuberk.* **4** (1987) 44–45. (in Russian)

Tuberculosis epidemiological status in West Siberia was analyzed. Relations between epizootic unfavorable conditions and incidence of tuberculosis in man were revealed. In particular, the evidence of the role of bovine tubercle bacilli in development of tuberculosis in man and of possible reinfection of man and animals were presented.—Authors' English Abstract

Tsukamura, M., Mizuno, S., Toyama, H. and Ichiyama, S. Comparison of *in vitro* antimycobacterial activities of ansamycin and rifampin. *Kekkaku* **61** (1986) 497–503. (in Japanese)

In vitro antimycobacterial activities of ansamycin and rifampin were compared using Ogawa egg medium and the following conclusions were obtained. The *in vitro* growth-inhibitory activity of ansamycin against *Mycobacterium tuberculosis* strains was 2 to 4 times more active than that of rifampin. Rifampin-resistant mutant strain isolated from H37Rv strain of *M. tuberculosis* was 160 times more resistant to rifampin than the parent H37Rv strain, and it was 128 times more resistant to ansamycin than the parent strain. The responses to ansamycin rifampin-resistant strains isolated from patients who received administration of rifampin were different from strain to strain. Four of eight strains were resistant to 40 μg of ansamycin per ml but the other

four were less resistant and one of the four was susceptible to 1.25 μg of ansamycin per ml. The responses of rifampin-resistant *M. avium*-complex strains, the resistance of which was achieved without exposing to rifampin, were quite different from the response of *M. tuberculosis* strains that achieved their resistance by the administration of rifampin. Of 40 strains tested, 32 strains (80%) showed natural resistance to rifampin (40 $\mu\text{g}/\text{ml}$ or more). Of these 32, 19 strains (59%) were susceptible to 1.25 μg of ansamycin per ml. The natural resistance to rifampin did not always accompany the resistance to ansamycin. The *M. avium*-complex strains were frequently resistant to rifampin (80% of the strains were resistant to rifampin), but 27 (67.5%) of them were susceptible to ansamycin of 1.25 $\mu\text{g}/\text{ml}$. Although the relationship between the serotype and the susceptibility to ansamycin was not clearly demonstrated, strains with the serotypes 4, 8 and 16 were often susceptible to ansamycin. Ansamycin was more active than rifampin against *M. tuberculosis*, *M. bovis*, *M. kansasii*, *M. marinum*, *M. xenopi*, *M. haemophilum*, *M. scrofulaceum*, *M. nonchromogenicum* and *M. terrae*. Marked difference was observed in the activities to

M. marinum, *M. xenopi* and *M. haemophilum*.—Authors' English Abstract

Yurin, B. L. and Smirnova, E. Y. [Preparation of monoclonal antibodies to mycobacterial antigens.] *Probl. Tuberk.* **6** (1987) 64–67. (in Russian)

The basic parameters of the hybridoma technology for preparation of monoclonal antibodies to mycobacterial antigens were determined. The main attention was paid to development of procedures for animal immunization with complex antigens, identification of antibody-forming clones, and antibody testing. The optimal periods for the mouse immune response to tuberculosis antigens were chosen, the hybrid clones with the highest productivity of monoclonal antibodies to mycobacterial antigens were selected, a panel of test antigens was compiled, and the prepared antibodies were characterized by their ability to react with various mycobacterial antigens. A standard technology for preparation and characterization of monoclonal antibodies to various separate mycobacterial antigens was developed.—Authors' English Abstract