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CURRENT LITERATURE

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

General and Historical

Sehgal, V. N., Jain, M. K. and Srivastava, G. Evolution of the classification of leprosy. Int. J. Dermatol. **28** (1989) 161–167.

There is no universal consensus about leprosy classification. The Madrid and the Indian Leprologists' classifications, however, meet the essentials of clinical criteria, the prime denominator of intriguing dialogue. Nevertheless, the five-group classification has its protagonists in teaching and research institutions, because it was formed on the backdrop of clinical, bacteriologic, histopathologic, and immunologic characteristics. It is fitting to enlarge this classification by including indeterminate and pure mononeuritic/polyneuritic in its spectrum, making it an acceptable standard classification.—Authors' Conclusions

Sheela, G. R., Gopa, A. K., Revankar, C. R. and Ganapati, R. Leprosy teaching in medical colleges in Bombay—a questionnaire study. Indian J. Lepr. **61** (1989) 233–237.

An attempt was made to study the adequacy of leprosy teaching at the undergraduate level of the four medical colleges in Bombay, and to suggest possible routes toward the reorientation of leprosy teaching. Over 55% of the medical faculty contacted expressed dissatisfaction with the existing pattern of leprosy teaching. The survey reveals ample evidence pointing to the necessity of redesigning the curriculum at the undergraduate level, so as to provide increased weight to both the theoretical and the practical aspects of leprosy. A heartening feature of the study is the inclination shown by a majority of medical teachers to associate themselves with the Preventive and Social Medicine Department in order to help improve leprosy teaching and thereby help in leprosy control. This offer should definitely be taken advantage of for furthering the cause of leprosy eradication as a part of achievement of "Health for All by 2000 AD."—Authors' Abstract

Turk, J. L. and Rees, R. J. W. AIDS and leprosy. (Editorial) Lepr. Rev. **59** (1988) 193–194.

The editorialists discuss possible implications of HIV infection in their patients for leprosy workers. There may be an increased incidence of multibacillary compared with paucibacillary disease than previously because of "downgrading" on the disease spectrum due to depressed host immunity. HIV infection might interfere with response to leprosy chemotherapy. Active leprosy might recur, manifested as erythema nodosum leprosum. Subclinical leprosy could become apparent as tuberculoid leprosy in HIV-infected individuals; younger age at death could reduce the detection rate for lepromatous leprosy.

Regarding the on-going leprosy vaccine trials the editorialists caution that those vaccines involving live BCG should not be given to symptomatic HIV-infected individuals because of the risk of dissemination of the BCG. There is, however, as yet no evidence for withholding such vaccines from HIV-infected subjects without clinical disease. In view of the known immunosuppressive effect of HIV infection interpretation of the vaccine trial results would be incomplete unless representative trial candidates (and particularly those developing leprosy) have been tested for HIV status. The ethical need to counsel for HIV-testing would add a further strain on resources.—C. A. Brown (Trop. Dis. Bull.)

Chemotherapy

Achenbach, R. E. *Experience on mixed and supervised treatment of leprosy on the basis of a multidrug regimen proposed by the World Health Organization (WHO).* (Doctoral thesis in Medicine, University of Buenos Aires). Rev. Argent. Dermatol. **70** (1989) 58–59.

Since 1977, when I entered the Department of Dermatology after a short but significant stay in the then so-called Sanatorium Colonia Dr. Baldomero Sommer, my attention was drawn by the huge difference between the patient in the "leprosarium" and the outpatient, as well as the ignorance and fear of the disease from the majority of the physicians. After attaining some training on bacteriologic indexes (BI), I decided in 1983 to study the fitness of the regimen of combined therapy (MDT) proposed by WHO in 1981.

Objective: The presentation of the experience between 1983 and 1986 on a therapeutical approach with no deepening into other work lines as: reactions, vaccines, immunotherapy and/or experimental leprosy. The whole material was obtained from the Hansen Section of the Department of Dermatology. All available resources were employed: clinics, bacteriology [including bacteriological index (BI) and morphological index (MI), histopathology and immunology (Fernández and Mitsuda)].

Methods: Job and Chacko ranking was used (Ridley and Jopling modified), by suppressing BB (borderline-borderline), but keeping in mind the indeterminate leprosy (HI) which is adjusted to divide them into paucibacillary (PB): HI, borderline-tuberculoid (BT) and tuberculoid-tuberculoid (TT) with a BI $\leq$ from 2; and the multibacillary (MB): polar lepromatous (LL), borderline lepromatous (BL). The ranking was according to the initial clinical stage on the basis of a clinical, bacteriologic and histopathologic study (following WHO pattern). Previous therapies, number and intensity of reactions and former fulfillment were taken into account. The new scheme was carefully explained to each patient enhancing the importance of its acceptation; clofazimine (CLO) intake and regularity in monthly attendance.

Evaluation: Clinical: monthly in PB and MB; bacteriologic: previous to the treatment and every 3 months including at least four smears: nasal mucus, lesion and both auricle lobes, not less than 30 fields were examined by smears; Mitsuda: previous and at the end of MDT; histopathology: previous and every 6 months in PB and every 12 months in MB. Even though it is not required by WHO and as an original contribution, I carried out MI in MB patients. Medicines were provided by the Public Health Ministry and monthly survey was performed by a physician from the Leprosy Section. Therapeutic Scheme = MB: DDS (dapsone) 100 mg/d or 50 if the patient weighed less than 50 kg. CLO: 50 mg/d both self-administered (daily), plus 600 mg/mo. of RFP (rifampin) and 300 mg of CLO, supervised (monthly). As a minimum 2 years or until both bacteriology and biopsy do not exhibit bacilli (AFB) about 6–7 years in the LL with a large bacillary load. PB: DDS, 100 mg/d self-administered plus 600 of RFP monthly and supervised. Minimal duration of 6 months. In withdrawing the therapy UOWT (under observation without any treatment).

Results and conclusions: Fifty MB (14 BL and 36 LL) and 42 PB were assessed: 10 HI, 20 TT and 12 BT; 80% of MB fulfilled minimum of 2 years of MDT and most of them continued under therapy; none is given RFP as a monotherapy. Clinical, bacteriologic and histopathologic outcomes were successful, as well as the lower incidence of reactions (number and intensity). Two years are not enough time to negativize the smears in LL, for which I propose a histopathologic rather than a bacteriologic criterion or a minimum 7 years before discontinuing MDT, mainly in young patients. The huge bacillary destruction (bacilli just became powder) was outstanding and not seen before, even with daily RFP. In general, tolerance and acceptance were excellent with little rejection of pigmentation by CLO (physician-patient relationship?). PB: Only one patient could not complete the 6-month

therapy, that is, 97.6% were given the supervised doses, with excellent outcome in all parameters, including an increase of mm in lepromin (HI and BT). We only had 2 RR (reversal reactions) which were controlled by corticosteroids with no neurologic sequelae. Post-withdrawal MDT control in some PB reaches 6 years.

In considering the high resistance indexes to DDS, MDT should be accepted and, in fact, it is in all countries where leprosy is endemic. The scheme proposed by WHO experts, even though it can well be fit to characteristic features belonging to each country in agreement with their particular infrastructure and/or can be improved with new drugs, has yielded successful results in our experience. It is of interest that MB were not discharged until we were informed about the experience in centers with a large number of patients, or when biopsy exhibits residual lesions, or when MDT is in its 7th year in MB. RR should not be feared but treated appropriately. Old controversy concerning daily, weekly or monthly RFP has no further grounds to exist, daily doses do not improve in efficacy compared to monthly ones (600 mg/monthly is the correct course).—Author's Summary

Chattopadhyay, S. P., Gupta, C. M., Bhate, R. D., Bhate, R. P. and Sreevatsa. Indian J. Lepr. **61** (1989) 196–205.

Fifty-three multibacillary leprosy cases were treated with two regimens of MDT: L1 consisting of rifampin, dapsone and ethionamide and L2 consisting of rifampin, dapsone and clofazimine. The results were compared at regular intervals and at the end of the study (24 months). Clinical inactivity, bacteriological negativity, ENL reactions, upgrading reactions were seen in the L1 group in 65%, 4.54%, 50%, and 41% of cases, respectively, while 65%, 25.8%, 30% and 45%, respectively, in the L2 regimen group. Zero percent morphological index was achieved in all cases in the L1 regimen; 90% in L2 regimen cases. No viability was found on mouse foot pad inoculation after 6 months in L1 or after 18 months in L2 cases.—Authors' Abstract

Chopra, N. K., Agarwal, J. S. and Pandya, P. G. Impact of multidrug therapy on leprosy in Baroda District (Gujarat). Indian J. Lepr. **61** (1989) 179–189.

Although much information is available on multidrug therapy (MDT) organizing, it poses a challenge to the field staff due to limited field trials conducted and varied field conditions. The MDT project was begun on 11 June 1984 in Baroda by the government of India with the active assistance of the state government, World Health Organization, and Swedish International Development Agency. The drug combinations for multibacillary (MB) cases were rifampin (RMP) 600 mg, clofazimine (CLO) 300 mg, and dapsone (DDS) 100 mg daily for 14 days intensive supervised therapy followed by once a month (pulse) supervised doses of RMP 600 mg, CLO 300 mg, and DDS 100 mg for a minimum period of 2 years or more if indicated and CLO 50 mg daily with DDS 100 mg daily unsupervised for a minimum period of 2 years or more if indicated; for paucibacillary (PB) cases, DDS 100 mg daily for 6 months along with RMP 600 mg supervised (pulse) once a month for a minimum period of 6 months or more if indicated.

Total number of active cases at commencement of MDT was 10,706; out of these, 10,348 (96.37%) were brought under MDT until December 1987. Among 10,348 old active cases, 9112 (88.05%) were cured with MDT (3110 MB + 6002 PB). In 180 old MB cases, the BI is still positive in spite of 24 completed pulses. The remaining 1056 (10.23%) stopped treatment for various reasons; 790 (74.81%) cases have left the villages to earn their livelihood; 30 (2.86%) were being treated by skin specialists; and 86 (7.19%) refuse to continue treatment in spite of the best efforts of the field staff.

New cases detected from June 1984 until December 1987 totaled 7628, of which 7549 (96.28%) were brought under treatment. New cases cured until December 1987 are 4640 (1120 MB + 3520 PB); 17 cases relapsed after MDT (15 PB + 2 MB); 280 (1.56%) cases got complications; 250 developed reactions, 3 cases jaundice, 15 cases gastritis, and the remaining 12 got moderate to severe anemia. The prevalence rate came down from 5.81% to 1.01% per thousand population until December 1987. The de-

formity rate came down among new cases from 6.15% to 1.50% until December 1987. Regularity of treatment among PB cases was 90.84% compared to 90.48% for MB cases. The study showed that MDT can be implemented in tribal, rural, and urban populations with high rates of compliance.—Authors' Abstract

Choudhury, A., Mistry, N. F. and Antia, N. H. Effects of a derivative of serotonin (deoxyfructoserotonin) and other antileprosy drugs on attachment and uptake of *Mycobacterium leprae* by Schwann cells *in vitro*. Antimicrob. Agents Chemother. **33** (1989) 866–870.

The association (attachment and/or uptake) of *Mycobacterium leprae* with cultured Schwann cells was studied at 8 and 72 hr in the presence of a new antileprosy compound, deoxyfructoserotonin (DFS), as well as conventional antileprosy drugs such as rifampin (RFP) and 4, 4′-diaminodiphenyl sulfone (DDS). DFS significantly inhibited bacterial association with Schwann cells at 8 hr. RFP also affected the association of *M. leprae* but not to the same extent as DFS. A similar inhibition at 8 hr was noted when *M. leprae* but not Schwann cells were pretreated with DFS or RFP for 5 days before infection of cultures, implying that modulation was achieved through some form of drug action on bacteria. DDS had no effect on *M. leprae* association; however, the combination of DFS and DDS was neither antagonistic nor additive. At 72 hr postinfection, when attached but noninternalized bacteria were removed with trypsin-EDTA from Schwann-cell cultures containing DFS or RFP, a 50% reduction in the number of bacteria in the drug-treated group was obtained as compared with the numbers in drug-free cultures. This indicated a slow entry of *M. leprae* into Schwann cells in the presence of these drugs. Collectively, these observations point to differing requirements for late and early association of *M. leprae* with Schwann cells, besides suggesting a role for DFS and RFP in the prevention and minimization of *M. leprae*-induced nerve damage *in vivo*.—Authors' Abstract

Holdiness, M. R. Clinical pharmacokinetics of clofazimine; a review. Clin. Pharmacokinet. **16** (1989) 74–85.

Clofazimine is useful in the treatment of Hansen's disease (leprosy) and some dermatological disorders, and is currently being used in drug regimens for patients with human immunodeficiency viral infections who are also infected with *Mycobacterium avium* complex.

After an oral dose, absorption is variable, but when given in an oil-wax suspension is approximately 70%. Administration with food appears to increase the peak plasma drug concentration and reduce the time to peak level. Data on the volume of distributior and percentage or type of protein bindi , are not available; however, the drug unde oes extensive tissue distribution. Clofazimine does not cross the blood-brain barrier, but does cross the placenta, and is found in human breast milk.

To date, three urinary metabolites have been identified in man, but their biological activity is unknown. A substantial portion of the unchanged drug is excreted in feces. The elimination half-life is variable, with values as long as 70 days being quoted in the literature.

Frequently reported side effects of clofazimine are hyperpigmentation of the skin and conjunctiva, and abdominal pain. These resolve upon cessation of therapy. Biochemical and hematological adverse effects have been reported, but are generally not clinically relevant. Pharmacokinetic drug interactions of potential clinical significance have been observed with dapsone, estrogen, rifampin and vitamin A.—Author's Summary

Mester de Parajd, M., Ambrose, E. J., Tayeb, N. and Antia, N. H. [La desoxyfructoserotonine: son effect thérapeutique dans le traitement de la lèpre.] Acta Leprol. **7** Suppl. 1 (1989) 200–202. (in French)

Desoxyfructo-serotonin (DFS) has shown good results in clinical trials of LL patients. After clinical trials in Bamako (Mali) reported in three articles, clinical trials began in India, at Bombay. Acute toxicity tests done in Paris and chronic toxicity tests done in India had shown absence of side effects.

This was also confirmed after pre-clinical pharmacology. *In vitro* tests show that DFS enhances cellular immune response. Receptors for anti-erythrocyte antibody on LL macrophages are demonstrated by erythrocyte rosetting. Infection with *M. leprae* markedly reduces rosetting, but in the presence of DFS this reduction in rosetting is not observed. Patients' peripheral blood lymphocytes, sensitized with leprosy antigen, show a low level of rosetting with patients' macrophages. DFS greatly enhances the lymphocyte-macrophage interaction. DFS has an important anti-stress activity. Gastric ulcer induced in rats by restraint were reduced by 40% and 50%. DFS increased the uptake of serotonin by LL patients platelets. HPLC studies were done to see the level of DFS in the plasma, in the serum and the urine of LL patients and controls. We are synthesizing new liposoluble derivatives in order to make the penetration of DFS easier and to prolong its duration of action.—Authors' English Summary

Parking, A. A. and Shah, B. H. Flu like syndrome with rifampicin pulse therapy. Indian J. Lepr. **61** (1989) 209–210.

Flu-like syndrome was found in a patient with BT leprosy taking rifampin in pulse therapy. This side effect was absent when the dose of rifampin was decreased. Details of this case are given with a review of literature.—Authors' Abstract

van Brakel, W., Kist, P., Noble, S. and O'Toole, L. Relapses after multidrug therapy for leprosy: a preliminary report of 22 cases in West Nepal. Lepr. Rev. **60** (1989) 45–50.

The World Health Organization-recommended multidrug therapy (MDT) regimens for leprosy patients were implemented in Nepal from 1982. Therefore a considerable number of both paucibacillary anld multibacillary patients have been on observation after release from MDT for as long as 4–5 years. A retrospective study was done considering the patients who relapsed during this period and who were registered at the outpatients department of Green Pastures Hospital in Pokhara, Nepal. A total of 22 patients relapsed out of 927 who were released from MDT.—Authors' Summary

van Saane, P. and Timmerman, H. Pharmacochemical aspects of leprosy; recent developments and prospects for new drugs. Pharm. Weekbl. [Sci.] **11** (1989) 3–8.

From a pharmacochemical point of view the existing antileprotics as well as possible innovations in the chemotherapy of leprosy are discussed. Of the main antileprotics, which are used nowadays—dapsone, rifampin, clofazimine, isoniazid, ethionamide and prothionamide—the mechanism of action, the main problems in their application and possibilities to develop improved variants are reviewed. Based on the chemistry of *Mycobacterium leprae*, the target systems for new antileprotics are identified. These systems include the cell wall, the catabolism of reactive oxygen species, the metabolisms of carbon sources, the amino acid metabolism and the uptake of iron. Two possible new lead structures from other fields, 4-quinolones and mycobacterial ribonucleotide reductase inhibitors, are presented.—Authors' Abstract

Clinical Sciences

Arora, S. K., Mukhija, R. D., Mohan, L. and Girdhar, M. A study of cutaneous lesions of leprosy on male genitalia. Indian J. Lepr. **61** (1989) 222–224.

The cutaneous lesions of leprosy on male genitalia were studied. They were found in 2.9% of cases examined in this series. They were seen most commonly in the borderline group. It is emphasized that it is not uncommon to find lesions on male genitalia in leprosy.—Authors' Abstract

Arora, S. K., Mukhija, R. D., Mohan, L., Girdhar, M. and Sharma, S. P. A study

of palmo-plantar lesions in leprosy: a preliminary report. Indian J. Lepr. **61** (1989) 206–208.

Out of 500 leprosy patients screened for palmar and/or plantar lesions, 18 cases were detected. They were classified according to the Ridley-Jopling classification. In the majority of cases it was a macular lesion. Cases were from TT, BT and BB groups. In 50% of the cases, palmo-plantar involvement was associated with type 1 reaction. In 66.7% of the cases, it was an extension of a patch from a surrounding area; in 11.1% of the cases isolated lesions were seen and in 22.2% of the cases both extension as well as isolated lesions were present.—Authors' Abstract

Atkin, S. L., El-Ghobarey, A., Kamel, M., Owen, J. P. and Dick, W. C. Clinical and laboratory studies of arthritis in leprosy. Br. Med. J. **298** (1989) 1423–1425.

Arthritis associated with leprosy is underreported. In Egypt 66 patients from a leprosy colony were studied, 20 of whom had arthropathy. This was characterized by an inflammatory symmetrical peripheral polyarthritis. The wrist, metacarpal and proximal interphalangeal joints of the hands, the knees, and the metatarsophalangeal joints of the feet were affected, with associated morning stiffness. The arthritis was erosive in 11 out of 20 patients, had no features of the arthritis associated with erythema nodosum leprosum reactions, but symptomatically responded to antileprosy treatment. This arthritis would seem to be a previously unrecognized feature of leprosy.—Authors' Abstract

Balybin, E. S. [Thyroid hormones in leprosy patients.] Vestn. Dermatol. Venereol. **8** (1988) 48–51. (in Russian)

The blood plasma triiodothyronine (T_3) and thyroxine (T_4) levels have been measured in 137 patients with lepromatous and borderline lepromatous leprosy, and the hypophyseal thyrotropic hormone (TTH) level, in 114 such patients. No cases of primary endocrine disorders have been recorded in these groups, and no endocrine therapy has been administered to these patients. The mean T_3 levels in leprosy have been significantly elevated versus the norm and, in contrast to the norm, these levels correlated with a significant rise of TTH level. Another pattern has been observed in cases of borderline lepromatous leprosy during stable regression: T_3 levels have been reduced to the norm; whereas TTH level has been still higher. T_4 concentrations in all the patients virtually did not differ from those in healthy subjects and did not depend on the disease activity. Noticeable seasonal fluctuations of T_3 and TTH levels have been recorded: the concentrations tended to rise in spring and more so in winter. The author suggests a possible relationship between thyroid function and the development of leprosy relapses.—Author's English Summary

Chattopadhyay, S. P., Rajpathak, S. D., Gopal, A. R. and Patra, A. K. Bilateral involvement of sole and palm in leprosy; a case report. Indian J. Lepr. **61** (1989) 266–267.

A case of BT leprosy in reaction with lesions over uncommon (immune) sites like the palm and the sole is reported.—Authors' Abstract

Chattopadhyay, S. P., Rajpathak, S. D., Rajagopal, A. and Patra, A. K. Multiple synovial swellings in BT leprosy; a case report. Indian J. Lepr. **61** (1989) 263–265.

A case of leprosy with multiple synovial swellings has been reported. Rarely these swellings may be an initial presentation and at sites other than the dorsum of hand or the ankle as was in this case. The literature on the subject is briefly reviewed.—Authors' Abstract

Mehrotra, S., Dhiman, R. K. and Sircar, A. R. Hyper-eosinophilic syndrome with lepromatous leprosy. J. Assoc. Physicians India **36** (1988) 287–288.

A case of persistent hypereosinophilia with organomegaly and hepatic and bone marrow infiltration with eosinophils associated with treated lepromatous leprosy is reported. There was a good response to prednisolone therapy.—Authors' Summary

Nilsen, R., Mengistu, G. and Reddy, B. B. The role of nerve biopsies in the diagnosis

and management of leprosy. Lepr. Rev. **60** (1989) 28–32.

Skin and nerve biopsies from 81 patients clinically suspected to have leprosy were studied. Histologically 54% of the patients showed leprosy. Both nerve and skin biopsies were histologically diagnostic of leprosy in 64% of these cases while 32% were diagnostic in the nerve but not skin biopsy. In the 11 patients with multibacillary leprosy (BI $\geq$ 2) a multibacillary picture was seen in all nerve biopsies while 8 patients exhibited a paucibacillary leprosy of the skin and a multibacillary leprosy in the nerve. The present results emphasize that leprosy is a disease of peripheral nerves and that diagnostic criteria other than skin parameters are important to reach a proper diagnosis. The evident possibility of having patients with multibacillary leprosy in peripheral nerves and paucibacillary in skin emphasizes the need of clinical studies to clarify the criteria for the diagnosis of paucibacillary leprosy and the drug regimen for this group of patients.—Authors' Summary

Ponnighaus, J. M. and Fine, P. E. M. A comparison of sensory loss tests and histopathology in the diagnosis of leprosy. Lepr. Rev. **60** (1989) 20–27.

Three different sensory loss tests, for anesthesia to light touch, for diminished pain sensation and for loss of thermosensation, were compared with histopathological examination results in the diagnosis of suspected tuberculoid leprosy in 120 individuals with 126 lesions. Although none of the three tests used in this study was found to be strikingly superior to any of the others, the results indicate potentially important differences in their usefulness in different subgroups of suspected patients. The methodological problems inherent in such studies are discussed.—Authors' Summary

Raman, J. and Fisher, D. A florid case of borderline-lepromatous leprosy. Med. J. Aust. **149** (1988) 701–702.

A case of borderline-lepromatous leprosy is presented to highlight the detection and prevalence of florid, new cases of leprosy toward the lepromatous end of the spectrum in the northern and western regions of Australia; the recognition and early treatment of lepra reactions; and the potential use of an enzyme-linked immunosorbent assay to detect and to monitor immunoglobulin M levels in leprosy.—Authors' Abstract

Soshamma, G. and Suryawanshi, N. Eye lesions in Leprosy. Lepr. Rev. **60** (1989) 33–38.

Out of 742 outpatients screened for ocular disease, 177 (24%) had eye lesions due to leprosy. These were more in the lepromatous spectrum of the disease showing an increasing trend with age of patient and duration of the disease. Madarosis was the commonest lesion (76%). The serious and sight-threatening lesions like lagophthalmos, corneal anesthesia, corneal opacities and ulcers, iritis and complicated cataracts constituted 8.22% of the lesions. Blindness due to corneal opacity and complicated cataract developed in 6 patients, constituting 3.4% of eye lesions with a prevalence rate of 0.8% among all the leprosy patients. Although the blinding lesions occurred in a very small percentage of patients, most of these are preventable through early recognition and institution of appropriate treatment. The simple techniques of examination to detect potentially sight-threatening lesions should be taught to all leprosy workers to prevent blindness among leprosy patients.—Authors' Summary

Tibrewala, K. D., Patel, T. K., Desai, A. R. and Rangwala, M. A. Tetanus with leprosy. J. Assoc. Physicians India **36** (1988) 395–396.

A known case of leprosy presenting with tetanus is reported because tetanus is rarely encountered in leprosy patients.—Authors' Summary

Vaishnavi, C., Sokhey, C., Kaur, S., Kumar, B., Dilawari, J. B. and Ganguly, N. K. Leprosy and hepatitis B surface antigen. Indian J. Lepr. **61** (1989) 211–215.

An ELISA technique has been developed to detect HBsAg in the sera of leprosy patients. Out of 92 serum samples taken from untreated leprosy patients, 10 samples were positive for HBsAg. The ELISA used in the present investigation is a low cost, reliable

and sensitive marker of HBsAg. It is better than less sensitive (hemagglutination and counterimmunoelectrophoresis), costly and hazardous (radioimmunoassay) techniques and is therefore recommended for routine use.—Authors' Abstract

Wong, M.-Y. and Chang, W.-K. Relationships between oro-facial lesions, mutilations and periodontal status in leprosy patients at Lo-Sheng Sanatorium at Taiwan. J. Formosan Med. Assoc. **87** (1988) 437–444.

Facial nerve paralysis with abnormal masticating forces and multilated hands or fingers have been stated as the causes of poor oral hygiene and the high prevalence of periodontitis among leprosy patients. The oral lesions anld periodontal conditions of 480 leprosy inpatients in the Provincial Lo-Sheng Sanatorium were evaluated and the contributing factors related to periodontal destruction were also discussed. Acute lesions within the oral cavity could not be detected because of the long-standing and well-conducted treatment. Early detection and treatment have reduced the incidence of late complications. Generalized gingival recession associated with mild or moderate gingivitis, minimal pocket formation and the absence of tooth mobility were found in the majority of cases. The inflammatory signs of the gingivae were not commensurate with the local irritative factors. Hand disability and intraoral lesions did not exacerbate the periodontal destruction of these patients. Local interferences, especially caused by facial nerve paralysis and atrophy of the nasomaxillary complex, aging, and lack of motivation to take care of their dental health, were the main causes of impairing their oral hygiene performance, being related to the progression of periodontal destruction. On the other hand, long-term medication of antibiotics and changes in underlying different host responses of these patients may contribute to reduce periodontal involvement. The results obtained from the present survey implicate that chemotherapy is a valuable method to control bacterial periodontal disease. Modification of the tooth-brushing technique, use of chemotherapeutic agents for rinsing, and encouragement of their motivation are the most important measures before any further dental service can be undertaken.—Authors' Abstract

Immuno-Pathology

Chanteau, S., Cartel, J.-L., Plichart, R., Roux, J. and Bach, M.-A. PGL I antigen and antibody detection in the control of leprosy in French Polynesia. Acta Leprol. 7 Suppl. 1 (1989) 128–129.

The results showed that PGL-I antigen (Ag) and anti-PGL I antibody (Ab) are poorly detected in the sera of PB patients, conversely to the sera of MB patients. The following up of the household contact population revealed that the Ab test is of poor predictive value but the PGL-I Ag test may reflect more closely the current multibacillary infection. Combined Ag and Ab tests in healthy contacts gave evidence that infection occurs frequently and is scarcely followed by apparition of the disease. Finally, the results suggest the possible usefulness of the PGL-I Ag test for monitoring MDT in MB patients.—(*From* the Article)

Cho, S.-N., Shin, J.-S., Choi, I.-H., Kim, S.-H., Kim, D.-I. and Kim, J.-D. Detection of phenolic glycolipid I of *Mycobacterium leprae* and antibodies to the antigen in sera from leprosy patients and their contacts. Yonsei Med. J. **29** (1988) 219–224.

Serum specimens from leprosy patients, their contacts, and controls were examined for the presence of phenolic glycolipid (PGL-I), a *Mycobacterium leprae*-specific antigen, and antibodies to the antigen using enzyme-linked immunosorbent assays. Of 12 lepromatous patients with less than 2 years of therapy, 11 (91.7%) were seropositive to

PGL-I, thus indicating that new lepromatous cases can be identified by detecting anti-PGL-I antibodies. In contrast, 88 (56.4%) of 156 lepromatous patients treated more than 2 years were positive. Moreover, only 69 (40.8%) were seropositive among 169 lepromatous patients in the leprosy resettlement villages. The mean antibody level also declined significantly in proportion to the duration of chemotherapy. This may suggest the possibility of monitoring chemotherapy by detecting anti-PGL-I antibodies. The prevalence of anti-PGL-I antibodies among 200 controls from a highly endemic area for leprosy was 5.5% and was significantly higher than that (1.5%) among 200 controls from a low-endemic area. Of 103 household contacts in the resettlement villages, 10 (9.7%) were seropositive, reflecting the frequent chance of exposure to *M. leprae.* However, PGL-I was not detected in any of the sera from controls, contacts, and inactive lepromatous patients having the anti-PGL-I antibodies; on the other hand, 6 (50%) of 12 lepromatous patients treated less than 2 years had detectable PGL-I in their sera. The results thus indicate that PGL-I detection may be more suitable for monitoring the effectiveness of chemotherapy and that it may be necessary to examine for the presence of PGL-I in sera from contacts and normal populations for confirming *M. leprae* infection.—Authors' Abstract

Das, P. K., Rambukkana, A., Bass, J. G., Groothuis, D. G., Kok, A. and Halperin, M. Identification of mycobacterial antigens for "ELISA" serology in the diagnosis of leprosy and tuberculosis. Acta Leprol. **7** Suppl. 1 (1989) 117–120.

Using an immunoblotting assay (ImBA), several immuno-crossreactive antigenic components (ImCRAC-myc) have been identified in the whole sonicates of *Mycobacterium bovis*-BCG, and *M. tuberculosis* (Mtb) and *M. leprae* (ML) whereby the sera of 100% lepromatous leprosy (L-Lep) reacted to 29/33 kDa doublet and that of 100% tuberculoid leprosy (T-Lep) reacted to 64-kDa bands. The antigens upon purification from Mtb sonicates were used in a direct ELISA to measure antibody isotypes in the sera from L-Lep, T-Lep, healthy leprosy contacts (Lep. c), normal Dutch controls (N), and tuberculosis (TB) patients. A significantly high IgG titer to the doublet 29/33-kDa and to 64 kDa were observed among L-Lep and T-Lep patients respectively in comparison to sera from other groups of individuals. In certain cases of L-Lep patients, raised IgM titer to either or both to 29/33 kDa doublet and 64 kDa were also found. On the other hand, consistently but significant high IgA-antibody titer to cell wall (CW), cytosol (cyt) and P_{90} fractions of Mtb distinguished clearly the TB patients from Lep groups, normals (NN) and Lep. c. It appeared that such antibody reactivity of TB sera might be directed to the groups of 58–60, 38–40, 18–20 and 14 kDa antigens of mycobacteria, e.g., Mtb. On the basis of the present observations, we conclude that the measurement of class-specific antibody response to the panel of these antigens could diagnose differentially between Lep, TB and NN/Lep. c among the population at large in an endemic area.—Authors' Summary

De Libero, G., Flesch, I. and Kaufmann, S. H. E. Mycobacteria-reactive Lyt-2^+ T cell lines. Eur. J. Immunol. **18** (1988) 59–66.

After re-stimulation *in vitro,* spleen or lymph-node cells from mice immunized with killed *Mycobacterium tuberculosis* or viable *M. bovis* were cytolytic for bone-marrow-derived macrophages pulsed with the same mycobacteria. T-cell lines with the phenotype Thy-1^+, L3T4^-, Lyt-2^+ were obtained by limiting dilution, and were also shown to be cytotoxic. The cytotoxicity of some of the lines was Class I restricted; whereas other lines showed antigen-specific killing without any H-2 restriction. None of the lines tested produced IL-2, but 5 of 8 lines secreted γ-interferon after stimulation with antigen. When supernatants from the lines were added to bone-marrow macrophages containing live *M. bovis,* [^{3}H]uracil uptake by the bacteria was reduced by > 80%, and this effect was blocked by the addition of antibody against γ-interferon. In contrast, other T-cell lines that failed to produce γ-interferon also inhibited the growth of the mycobacteria when added directly to the macrophage cultures.—H. M. Dockrell (Trop. Dis. Bull.)

Desforges, S., Launois, P., Bobin, P. and Bach, M.-A. Follow up of T cell subsets and anti-trisaccharide IgM antibody levels of leprosy patients during daily multidrug therapy. Acta Leprol. 7 Suppl. 1 (1989) 169–170.

T-cell subset distribution and anti-*Mycobacterium leprae*-specific antibody levels were followed in leprosy patients during polychemotherapy (or multidrug therapy, MDT). Before treatment, the T-cell subset counts showed that the multibacillary patients (28) have a significant decrease ($p < 0.05$) in the CD3+, CD4+ T percentages and CD4+/CD8+ ratio as compared to the healthy controls (96). The paucibacillary patients (30) had no significant T-cell disturbances. An ELISA test has been developed using the natural trisaccharide-octyl-BSA (NTO) antigen to identify specific IgM antibodies (IgM anti-T). Multibacillary patients (22) showed higher antibody titers than paucibacillary patients (11). But the IgM anti-T levels of the two groups are significantly higher than controls belonging to the same ethnic group. A daily polychemotherapy (dapsone, rifampin, clofazimine) was done for 6 months in paucibacillary patients and 2 years in multibacillary patients according to the leprosy group study recommendations. A normalization of the CD4+ T cells was obtained by a 6-month therapy in multibacillary patients and a significant decrease in the IgM anti-levels was obtained during polychemotherapy in multibacillaries ($p < 0.001$) and paucibacillaries ($p < 0.05$).—Authors' Summary

Douglas, J. T., Hirsch, D. S., Fajardo, T. T., Cellona, R. V., Abalos, R. M., de la Cruz, E. C., Madarang, M. G., de Wit, M. Y. L. and Klatser, P. R. Evaluation of *Mycobacterium leprae* antigens in the serological monitoring of a clofazimine-based chemotherapeutic study of dapsone resistant lepromatous leprosy patients in Cebu, Philippines. Lepr. Rev. **60** (1989) 8–19.

Thirty-one dapsone-resistant lepromatous leprosy patients receiving clofazimine-based therapy were serologically monitored throughout their 5-year period of treatment. Sequentially collected sera were used to examine four *Mycobacterium leprae* antigens to evaluate their usefulness in ELISAs for monitoring the progress of their therapy. The ELISA results were compared with decline in bacterial load over the treatment period and with duration of treatment. In addition the ELISAs were compared with each other. The ELISAs based on the measurement of IgM antibodies to the two neoglycoproteins (NDO and NTO) representing the phenolic glycolipid antigen of *M. leprae* were found to be the most effective with regard to monitoring treatment. A whole *M. leprae*-based ELISA was less efficient in monitoring treatment because it failed to measure antibodies in 5 out of 31 patients. The ELISA-inhibition test based on the detection of antibodies to a species-specific epitope on the 36 kDa antigen of *M. leprae* was less suitable because of persistent reactivity during therapy.—Authors' Summary

Huerre, M., Desforges, S., Bobin, P. and Ravisse, P. Demonstration of PGL I antigens in skin biopsies in indeterminate leprosy patients: comparison with serological anti-PGL I levels. Acta Leprol. 7 Suppl. 1 (1989) 125–127.

The survey of histological findings in leprosy patients from 1985 to March 1988 has been carried out at the Pasteur Institute in Noumea. Histologically, according to Ridley Jopling criteria, 82 patients were classified: 14 as TT, 12 as BT, 2 as BB, 7 as BL, 4 as LLs, 24 as LLp and 19 as indeterminate leprosy. Histological examination of tissue sections, using a monoclonal anti-PGL-1 antibody showed PGL-1 antigen in histiocytic cells of the infiltrate and more rarely in Langerhans' cells in 7 cases of indeterminate leprosy. The Ziehl staining method revealed the presence of alcohol resistant bacilli in only one case. For 10 patients, histological findings were compared with serological results. In 3 cases, the diagnosis was confirmed by the two techniques (immunohistology and serology). In 2 cases by only immunohistology or by serology and in 3 cases the diagnosis was not confirmed by either method. These results showed the interest of the immunohistological and serological methods in indeterminate leprosy, especially in children. A study of household

contacts may be of interest.—Authors' Summary

Janis, E. M., Kaufmann, S. H. E., Schwartz, R. H. and Pardoll, D. M. Activation of γδ T cells in the primary immune response to *Mycobacterium tuberculosis.* Science **244** (1989) 713–716.

Although the immunologic role of T cells bearing the conventional αβ T-cell receptor (TCR) has been well characterized, little is known about the function of the population of T cells bearing the γδ TCR. Therefore, the role of γδ T cells in the immune response to *Mycobacterium tuberculosis* (MT) was investigated. The number of TCR γδ cells in the draining lymph nodes of mice immunized with MT was greatly increased in comparison with the number of TCR αβ cells. Three biochemically distinct γδ TCRs were detected. Analyses of cell cycle, of interleukin-2 receptor expression, and of interleukin-2 responsiveness showed that a large proportion of the γδ T cells were activated *in vivo.* TCR γδ cells responded to solubilized MT antigens *in vitro* but, in contrast to MT-specific αβ T cells, the response of γδ T cells to MT did not require major histocompatibility complex class II recognition. These results provide an example of antigen-specific activation of γδ T cells *in vivo* and indicate that γδ T cells may have a distinct role in generating a primary immune response to certain microorganisms.—Authors' Abstract

Katoch, K., Natrajan, M., Narayanan, R. B. and Katoch, V. M. Immunotherapy of treated BL/LL cases with BCG: histopathological, immunohistological and bacteriological assessments. Acta Leprol. 7 Suppl. 1 (1989) 153–155.

The persistence of dead as well as viable bacteria is an important therapeutic problem in multibacillary leprosy. In highly bacillated patients, viable bacteria are detectable in 10%–15% of cases after 2 years of treatment, and none of these cases became smear negative by 2 years of recommended multidrug therapy (MTD). Immunotherapy trials using BCG (0.1 mg) by intradermal route have been undertaken in cases who had MDT for 2 years and who had viable bacilli by ATP photometry and/or FDA-EB staining. Biopsies and smears were taken from local as well as distant sites at 0.4 weeks and 6 months after BCG vaccination. Biopsies were processed for ATP counts, FDA-EB staining, histopathology and immunohistology for cell types at 0.4 weeks. There was transient effect on BI, ATP counts, FDA-EB staining at local as well as distant sites in some cases. Histopathology and immunohistological findings suggest that there is a tendency to form epitheloid cell granuloma at the local site in all cases and at distal sites in some. There was infiltration of subepidermal zone in one case 4 weeks after vaccination. BCG may be of use as a potential immunotherapeutic agent but its usefulness needs to be investigated in depth preferably in the beginning or early phases of chemotherapy and also with repeated inoculations.—Authors' Summary

Kaufmann, S. H. E., Golecki, J. R., Kazda, J. and Steinhoff, U. T lymphocytes, mononuclear phagocytes, Schwann cells and *Mycobacterium leprae.* Acta Leprol. 7 Suppl. 1 (1989) 141–148.

It is the purpose of this article to discuss recent studies performed in the mouse system aimed at dissecting the relationship between T lymphocytes, mononuclear phagocytes, Schwann cells, and *Mycobacterium leprae.* The data indicate: (1) Besides CD4 T cells, also CD8 T cells participate in the immune response to *M. leprae*; (2) both T cell sets produce interferon-γ (IFN-γ), a potent macrophage activator; (3) tumor necrosis factor-α (TNF-α) is involved in necrosis in leprosy granulomas; (4) CD8 T cells lyse macrophages presenting *M. leprae* antigens; (5) in *M. leprae*-infected Schwann cells lysosomes fuse with phagosomes; (6) Schwann cells presenting *M. leprae* antigen are lysed by CD8 T cells.—(*From* the Article)

Kaur, I., Vaishnavi, C., Kaur, S., Sharma, V. K., Ganguly, N. K., Kohli, M. and Wangoo, A. Lymphocytotoxins in leprosy. Indian J. Lepr. **61** (1989) 164–168.

Lymphocytotoxic antibodies (LCAs) were assayed in serum samples from 60 patients of the leprosy spectrum before starting mul-

tidrug therapy (MDT). Seventeen healthy volunteers without any history of viral infection provided control samples. Post-treatment follow-up samples were also included in the study. In all pretreatment sera of LL, BL and BT/TT patients the levels of LCAs were elevated. With treatment a significant fall in LCA production was observed in all types of leprosy patients.—Authors' Abstract

Kaur, S. and Abraham, A. Vascular involvement in leprosy. Indian J. Lepr. **61** (1989) 238–248.

Vascular involvement in leprosy is no more an ambiguous postulation, but a definite histopathological, ultrastructural and angiographic entity. It may play a role in the pathogenesis of the disease. From the endothelium, bacilli may continuously contaminate the blood stream; in the media they may persist despite many years of treatment. Vascular involvement may contribute directly or indirectly to dystrophic changes; and it provides food for thought regarding the possible mode of transmission through arthropods.—Authors' Conclusion

Khanolkar, S. R., Young, D. B., Brennan, P. J., Buchanan, T. M. and McAdam, K. P. W. J. Use of an antigen-capture assay for characterization of monoclonal antibodies to mycobacterial lipoarabinomannan. J. Med. Microbiol. **28** (1989) 157–162.

Monoclonal antibodies directed to six separate antigen molecules of *Mycobacterium leprae* have been tested in an antigen-capture assay based on combined use of polyclonal ("capture") and monoclonal ("detector") antibody reagents. This approach provides a potentially versatile, sensitive and specific assay for detection and relative quantitation of *M. leprae* antigens. Characterization of monoclonal antibodies to mycobacterial lipoarabinomannan (LAM-B) by the antigen-capture assay indicates that some of the antigenic determinants present on LAM-B from *M. leprae* may be either absent altogether or present at much lower concentrations on the corresponding LAM-B structure from *M. tuberculosis.*—Authors' Summary

Klenerman, P. Prostaglandins and leprosy. A role for aspirin? Lepr. Rev. **60** (1989) 51–58.

Prostaglandins not only have a role in inflammation, but may also be involved as mediators in the immune response. Drugs which affect prostaglandin synthesis may therefore be potential tools with which to modulate disturbed immunity. These possibilities are discussed with reference to immunity in leprosy, and in particular reversal reactions.—Author's Summary

Launois, P., Maillere, B., Dieye, A., Sarthou, J.-L. and Bach, M.-A. Limiting dilution of murine T cell response to mycobacterial antigens. Acta Leprol. 7 Suppl. 1 (1989) 179–180.

This preliminary report shows marked crossreactivity at the T-cell level between the different mycobacteria studied in *Mycobacterium leprae,* BCG (IP-Dakar) and *M. fortuitum* immune mice. It suggests that the majority of reactive T cells respond to antigenic epitopes shared by all these mycobacteria. Limiting dilution analysis may be a useful technique to support a broadening specificity of the response or an enhancing reactivity in the administration of combined BCG/*M. leprae* vaccine.—(*From* the Article)

Liu, M.-F., Wang, C.-R., Chen, M.-Y. and Cheng, C.-S. Evaluation of cell-mediated immunity on leprosy patients in Taiwan: a preliminary report. J. Formosan Med. Assoc. **87** (1988) 622–624.

Leprosy patients, 22 tuberculoid (T) and 29 lepromatous (L), from Taiwan Provincial Lo-Sheng Leprosarium were studied for evaluation of cell-mediated immune status. Twenty-seven age- and sex-matched healthy individuals were included as a control group. All blood samples were subjected to immunological tests including lymphocyte subpopulations and lymphocyte responses to polyclonal activators. The values of all the lymphocyte subsets (CD3, CD4 and CD8) and lymphocyte responses to phytohemagglutinin (PHA) and concanavalin A (ConA) in leprosy patients, T type or L type, were lower than those in the control group

($p < 0.0001$ for CD3 and CD4, $p < 0.005$ for ConA, $p < 0.05$ for CD8 and PHA); however, the lymphocyte response to pokeweed mitogen (PWM) was preserved in leprosy patients. The L-type patients had a lower mean value of CD4 lymphocyte subpopulation and decreased response to PHA and ConA than the T-type patients ($p < 0.05$). The immunological status of leprosy was well correlated with the clinical features. The impairment of cellular immunity, much more severe for L-type patients, with the defect of CD4 subset is the principal immunological defect in leprosy patients. Further studies are needed to correlate immunologic abnormalities with therapeutic results and the disease course.—Authors' Summary

Mariano, M. and Ferreira, C. S. A. BCG infection induces responsiveness to dead *Mycobacterium leprae* in hamsters; a Mitsuda-like reaction. Braz. J. Med. Biol. Res. **22** (1989) 81–84.

Hamsters inoculated with BCG become responsive to dead *Mycobacterium leprae.* The response is characterized by a typical perineural granulomatous reaction, observed 16 days after the injection of a suspension of dead *M. leprae* into the foot pad of animals previously sensitized with BCG.—Authors' Abstract

Mehta, R., Birdi, T. J. and Antia, N. H. Effect of *Mycobacterium leprae*-infected Schwann cells and their supernatant on lymphocyte neuroglia interaction. J. Neuroimmunol. **22** (1989) 149–155.

Since the resolution of neural lesions and subsequent nerve damage in leprosy must inevitably involve the participation of immune cells sensitized to mycobacteria, we have used the dissociated Schwann cell culture model to study the relationship between *Mycobacterium leprae*-infected Schwann cells and sensitized immune cells. Our earlier study on light and ultrastructural observations showed that on infection with *M. leprae,* the cytomorphology of Schwann cells remains unaffected, while degenerative changes suggestive of apoptosis are seen in extraneous lymphocytes which are subsequently phagocytosed by the Schwann cells. We now present additional evidence confirming that the phagocytosis of splenic cells by Schwann cells is indeed a two-step process. The first involves *M. leprae*-dependent cytotoxicity to splenic cells. This is followed by phagocytosis of these cells, which is a secondary and *M. leprae*-independent phenomenon. This finding has implications particularly on the weak inflammatory response observed in nerve lesions of a majority of lepromatous patients.—Authors' Summary

Mendez-Samperio, P., Lamb, J., Botham-ley, G., Stanley, P., Ellis, C. and Ivanyi, J. Molecular study of the T cell repertoire in family contacts and patients with leprosy. J. Immunol. **142** (1989) 3599–3604.

The specificity of lymphocyte proliferative responses of 22 family contacts and 7 patients with leprosy were analyzed using antigen (Ag) fractions from soluble extracts of *Mycobacterium leprae* and *M. tuberculosis.* Fractions 10–100 kDa m.w. from each extract were separated by SDS-polyacrylamide gel electrophoresis, electroblotted to nitrocellulose membrane, and solubilized for use in lymphocyte culture. The main immunogenic fractions for both contacts and patients had m.w. of 12,000 to 22,000, 35,000 to 40,000, and 65,000. Determinants which were either distinct or shared by the two extracts were active in each of the immunogenic fractions. Lymphocyte proliferation following stimulation with separated Ag was found also in 5 subjects who failed to respond to the whole soluble extracts. Stimulatory synthetic peptides were identified for the 65 kDa protein Ag. This technique has permitted the screening of the T-cell immune repertoire for the identification of the immunodominant Ag which merit further purification and molecular characterization.—Authors' Abstract

Modlin, R. L. and Rea, T. H. Immunopathology of leprosy granulomas. Springer Semin. Immunopathol. **10** (1988) 359–374.

Clearly, we can learn much from lesions. Investigation of T-cell phenotypes in lesions across the leprosy spectrum provides a

framework for studying patients undergoing immunotherapeutic vaccination in order to gain insight into the T-cell subsets required for overcoming unresponsiveness and activating macrophages to eliminate intracellular pathogens. The T-cell clones derived from leprosy skin lesions can be used to elucidate the range of antigens and epitopes of *Mycobacterium leprae* recognized by the T-lymphocyte repertoire in leprosy. These *in situ* and *in vitro* approaches to the study of cells in leprosy skin lesions provide new information not only about the immunology of leprosy, but of basic immunological mechanisms of tissue inflammation and immunoregulation in man.—Authors' Conclusion

Muliyil, J. and Thangavel, N. Status of multibacillary patients "lost to follow up." Indian J. Lepr. **61** (1989) 229–232.

Completeness of coverage with MDT is essential if one is to hope for a reduction in the incidence of leprosy in an endemic area. Patients who are lost to follow up are generally declared as "permanently left" (PL) and deleted from the known case register. A special effort was made to study the true status of multibacillary patients who had been declared "PL" in the leprosy control program area of the Christian Medical College, Vellore. Thirty-eight percent of the 40 patients followed up were found to be still residing in the same area; 40% of them were BI positive. The reasons for these erroneous deletions varied. The study shows that declarations of "PL" need to be verified carefully. Further, it was found that the present system of reporting may exaggerate the actual number of patients who have been lost to follow up.—Authors' Abstract

Rambukkana, A., Das, P. K., Chand, A., Bass, J. G. and Groothuis, D. J. Monoclonal antibodies to 33 kD protein of *Mycobacterium tuberculosis* with varying epitope specificities. Acta Leprol. **7** Suppl. 1 (1989) 113–116.

Five mouse monoclonal antibodies (McAb) 5D2, 5D5, 5F9, 3A8 and 3F2 against *Mycobacterium tuberculosis* (M. tb) 33 kDa protein have been produced. All the McAbs except one were of IgG1; whereas the 3F2 was of IgM isotype. The ELISA and immunoblotting analysis show that 5D2, 5D5 and 5F9 are reactive to only 33 kDa protein; whereas 3A8 and 3F2 are also crossreactive to 29 kDa and 29 kDa + 64 kDa respectively. ELISA inhibition assays using peroxidase labeled respective McAbs show that 5D2 and 5D5 recognize the same or overlapping epitopes; whereas the remaining three McAbs (5F9, 3A8 and 3F2) are reactive to different epitopes of the 33 kDa protein. These McAbs are crossreactive to the majority of mycobacterial strains other than M. tb including *M. leprae* (ML). However such reactivities may involve proteins of differing molecular sizes. Interestingly, all these McAbs react to 25 kDa and 29 kDa of ML. The immunoreactivities of these McAbs to 33 kDa appear to be directed to peptide epitopes but not to carbohydrate moiety. Both IgG and IgM antibody activities of lepromatous leprosy sera to 33-kDa protein are heterogeneous and directed to more than four epitopes. The potential use of these McAbs in studying the pathomechanism of leprosy is currently being investigated.—Authors' Summary

Ranade, A. and Mahadevan, P. R. A component of *Mycobacterium leprae* as a serodiagnostic tool for leprosy. Indian J. Biochem. Biophys. **25** (1988) 554–559.

It has been observed that the delipidified component of the cell wall of *Mycobacterium leprae* (DCW), when used as an antigen, can distinguish and show different binding ability to sera of lepromatous leprosy patients and of healthy normals or tuberculoid leprosy individuals. This was demonstrated by the ELISA technique. Long-term-treated (> 4 years), bacteriologically negative lepromatous individuals showed no antibody in the sera for DCW. The level of antibody to DCW declined in patients undergoing treatment, and this appeared to happen rather rapidly. This could be indicative of the reduction in the quantum of viable *M. leprae* as was demonstrated with patients who were followed through several months of chemotherapy. The test appears to be specific. It is possible that whole DCW or some antigens associated with it could function as a specific serodi-

agnostic reagent for lepromatous leprosy.—Authors' Abstract

Rees, A., Scoging, A., Mehlert, A., Young, D. B. and Ivanyi, J. Specificity of proliferative response of human CD8 clones to mycobacterial antigens. Eur. J. Immunol. **18** (1988) 1881–1887.

Human CD8 T lymphocyte clones (TLC) were generated from the pleural effusion of patients with tuberculosis using a protocol that required, in addition to antigen, coculture of purified CD8+ T cells, accessory cells, interleukin 2 (IL-2) and anti-CD3-Sepharose. The TLC obtained were stimulated by micobacterial soluble extracts in an IL-2-dependent and MHC class I-restricted manner. When antigen-responsive TLC were screened with extracts from the recombinant mycobacterial library they were found to respond to either the Y3125 (100-kDa) or the Y3111 (71-kDa) λgt11 clones. Polyacrylamide gel immunoblot analysis demonstrated that the CD8 TLC responded to fractions with the molecular mass range 27–45 kDa in the Y3125 lysogen and 60–90 kDa in the mycobacterial soluble extract. The specificity of TLC reactive with the Y3111 clone was confirmed using the 71-kDa antigen purified from the same lysogen. These TLC recognized sequences common to the 71-kDa protein derived from mycobacteria, *Escherichia coli* or a humàn cell line. Studies of three TLC using antigen-presenting cells of known genetic haplotype indicated that stimulation with both the Y3125 and the 71-kDa antigens was restricted by determinants encoded by HLA-B8.—Authors' Abstract.

Saito, H., Tomioka, H. and Sato, J. Purification of *M. leprae* with special reference to the effects of purified *M. leprae* vaccines and host macrophage cell functions. Jpn. J. Lepr. **56** (1987) 101–109.

Mycobacterium leprae purified by the method of Draper (IMMLEP Protocol 1/79) based on Percoll gradient centrifugation and aqueous two phase separation system and the method of Mori, *et al.* based on the Percoll gradient ultracentrifugation were compared for some of their biological activities. The integrity of purified leprosy bacilli was higher in the latter preparation than the former. When the vaccines prepared from *M. leprae* cells obtained by the two purification methods by heat treatment at 100°C were studied for their efficacy to modulate host macrophage cell functions, the two vaccines were able to potentiate the phagocytic and active oxygen generating abilities but unable to augment the antilisterial activity of the host macrophages by double injections 21 days and 4 days before harvest via subcutaneous and intraperitoneal routes, respectively, *M. leprae* vaccine obtained by Draper's method more markedly heightened the phagocytic activity of host macrophages than that prepared by Mori's method; whereas the inverse was the case for enhancement of the active oxygen-producing ability of the host macrophages.—AS (Trop. Dis. Bull.)

Silva, C. L. and Foss, N. T. Inflammation induced by a glycolipid fraction from *Mycobacterium leprae.* Braz. J. Med. Biol. Res. **22** (1989) 327–339.

The inflammatory properties of a glycolipid fraction isolated from human recovered *Mycobacterium leprae* were investigated. The inflammatory reaction induced in mouse lung by the inoculation of the glycolipid fraction absorbed to charcoal particles was characterized by a large influx of macrophages at various stages of maturation and of epithelioid cells around the particles. When injected as an aqueous emulsion into the foot pads of mice, the same fraction evoked a dose-dependent massive influx of mononuclear (MN) cells. The inflammatory reaction reached a peak at 6 days. The minimal effective dose of glycolipid was 0.1 μg. The kinetics of inflammatory cell migration was studied by total and differential counts of leukocytes that migrated to the peritoneal cavity of mice inoculated intraperitoneally with the glycolipid fraction. This fraction initially induced intense polymorphonuclear (PMN) migration, which was later reduced, with a simultaneous increase in MN cells. Adherent peritoneal cells (APC) incubated with glycolipid released one or more soluble factor(s) which induce active PMN and MN cell chemotaxis *in vivo* as well as *in vitro.*

Thus, the MN cells may be attracted to the site of glycolipid inoculation by factor(s) released through the interaction of macrophages with the glycolipid fraction. The present results demonstrate that a glycolipid containing trehalose and mycolic acid isolated from *M. leprae* reproduces some aspects of the fundamental lesion of leprosy.—Authors' Abstract

Suso, L., Nogueira, N., Vieira, L. M. M., Figueiredo, A. A., Porto, J. A. and Sarno, E. N. [Correlation between skin tests and the production of gamma-interferon in patients with hanseniasis.] Med. Cut. Ibero Lat. Am. **16** (1988) 193–196. (in Portuguese)

The responsiveness of 25 leprosy patients to an *in vitro* correlate of cell-mediated immunity (CMI) to *Mycobacterium leprae* antigen (the production of γ-IFN by peripheral blood mononuclear cells) was compared to their responses to skin tests routinely used to evaluate CMI to a number of antigens including *M. leprae*. The results indicate that among the polar lepromatous (LL) patients there was a clear correlation between low responses in the *in vitro* assay (γ-IFN production), negative skin test to the specific antigen (Mitsuda reaction) and low responses to skin test to a related antigen (PPD). There were no correlations in the other clinical groups with skin tests to other unrelated antigens.—Authors' English Summary

Torgal-Garcia, J., Papa, F. and David, H. L. Immunological response to homologous and heterologous phenolic glycolipid antigens in tuberculosis and leprosy. Acta Leprol. **7** Suppl. 1 (1989) 102–106.

The occurrence of IgM antibodies immunoreacting in an ELISA test with five phenolic glycolipids (PGL) antigens (PGL-Tb 1, from *Mycobacterium tuberculosis*; PGL-I, from *M. leprae*; PGL-K-I, from *M. kansasii*; mycoside G, from *M. marinum*; and mycoside B, from *M. bovis*), was examined in the sera of 46 tuberculous patients, 48 multibacillary leprosy patients, 40 paucibacillary leprosy patients, and in 134 healthy controls. The sensitivity (97.9) and the specificity (91.8) observed in tuberculous patients with the homologous antigen PGL-Tb 1 underlined the interest of this antigen for case finding in tuberculosis epidemiology. The sensitivity and the specificity observed in multibacillary leprosy patients, respectively, 91.7 and 91.8, and in paucibacillary leprosy patients, respectively, 35.0 and 91.7, confirmed the limited value of homologous antigen PGL-I for the serological case finding of leprosy patients in endemic areas with a strong incidence of paucibacillary leprosy forms.

The data obtained with the heterologous PGL antigens in tuberculosis and multibacillary leprosy serology were higher than those observed in healthy controls and in paucibacillary leprosy patients. However, the ELISA using the heterologous antigens was not useful in diagnosis of active tuberculosis or multibacillary leprosy forms. Healthy controls showed low immunoreactivity against PGL antigens, with the exception of mycoside B.—Authors' Summary

Microbiology

Bera, A. and Banerjee, A. Studies on lipids in mycobacterial cell wall: their important structure and function relating to pathogenicity and their biological activity. Indian J. Lepr. **61** (1989) 143–150.

The lipids cord-factor, mycosides and sulfolipids are supposed to be vitally linked with the pathogenicity of mycobacteria. In this paper an attempt has been made to clarify the understanding of the occurrence, organization, and possible interaction of the diverse lipids present in the mycobacterial cell wall and their possible structure and function.—Authors' Abstract

Bharadwaj, V. P., Katoch, V. M., Katoch, K., Sharma, V. D., Kannan, K. B., Datta,

A. K. and Shivannavar, C. T. Studies on energy synthesis in *M. leprae.* Acta Leprol. 7 Suppl. 1 (1989) 30–32.

ATP measurements have been earlier used to study the effect of various nutrients on the growth and multiplication of *Mycobacterium leprae.* In a preliminary study, we had observed that glycerol and asparagine stimulated the ATP synthesis by *M. leprae* but this was marginal and not sustained. We have extended the study to investigate the role of various environmental factors which could affect this ATP synthesis. It has been observed that ATP synthesis was better and sustained for a longer period, i.e., up to 2 weeks, if the *M. leprae* were incubated at pH 6–6.5 and at 30–33°C in the modified Dubos and Sauton's media. The pH and temperature above these values were suboptimal. It is concluded that temperature and pH are important factors for maintenance and synthesis of ATP by *M. leprae.*—Authors' Summary

Bhatia, V. N. and Rao, S. Morphology of *M. leprae* (?) in VS_3E medium—a preliminary communication. Indian J. Lepr. **61** (1989) 160–163.

In a previous attempted culture of *Mycobacterium leprae* in VS_2M medium non-acid-fast organisms were seen initially and acid-fast organisms appeared later. A drop of a sonicated suspension from a subculture of this was inoculated in VS_3E medium. The inoculum consisted mostly of acid-fast granules. The culture yielded pure growth of acid-fast organisms. Morphology typical of *M. leprae* could be seen only after the 60th day of culture.—Authors' Abstract

Chatterjee, D., Hunter, S. W., McNeil, M., Jardine, I. and Brennan, P. J. Structure and function of mycobacterial glycolipids and glycopeptidolipids. Acta Leprol. 7 Suppl. 1 (1989) 81–84.

Earlier work from this and other laboratories has revealed the presence within *Mycobacterium* spp. of three classes of glycolipid antigens which we have called the glycopeptidolipids, the lipooligosaccharides and the phenolic glycolipids. Representative structures of each from different species and sub-species have been proposed. More recently, new variants of these antigens and older structures have been analyzed by Fourier transform infrared, NMR, particularly at high temperatures, and, most notably, by fast atom bombardment and Californium desorption mass spectrometry. Extraordinary novelty and diversity were revealed, particularly at the distal nonreducing end of the oligosaccharide chains, marked by the presence of new branched-chain sugars, amino sugars and sugar acids. These epitopes and monoclonal antibodies to them have been used for the critical identification of mycobacteria. In addition, the pure antigens are the basis of specific serological tests for various mycobacterioses.—Authors' Summary

Clark-Curtiss, J. E. Benefits of recombinant DNA technology for the study of *Mycobacterium leprae.* Curr. Top. Microbiol. Immunol. **138** (1988) 61–79.

Use of recombinant DNA methodology has great potential for increasing our understanding of the basic physiology of *Mycobacterium leprae,* for permitting a means of deciphering the mechanism(s) whereby the organism achieves its very successful pathogenicity, for producing some practical reagents for earlier diagnosis of the disease, for designing effective new drugs for treatment of leprosy and, ultimately, for prevention of leprosy altogether. This is an exciting time to be working in the field of leprosy research, both from the point of personal satisfaction in gaining new information about a poorly understood pathogen and from the point of being able to contribute to the solution of a disease that has caused so much suffering throughout history.—Author's Conclusions

Gicquel-Sanzey, B., Moniz-Pereira, J., Gheorghiu, M. and Rauzier, J. Structure of pAL5000, a plasmid from *M. fortuitum* and its utilization in transformation of mycobacteria. Acta Leprol. 7 Suppl. 1 (1989) 208–211.

We have developed a gene cloning system for mycobacteria. Based on the nucleotide sequence determined for the *Mycobacterium fortuitum* plasmid pAL5000, we have constructed an *Escherichia coli*/mycobac-

teria shuttle vector. This vector, pAL8, is composed of pAL5000, pTZ19R (an *E. coli* plasmid) and a kanamycin-resistance gene (from Tn903). We were unable to obtain viable kanamycin-resistant pAL8 transformants of *M. smegmatis* using a PEG-mediated DNA uptake system, in spite of the fact that we could show efficient DNA uptake by transfection using the mycobacterial lytic phage D29. However, kanamycin-resistant transformants of *M. smegmatis* or BCG could be obtained by electroporation. This plasmid cloning system provides a tool for studies of the expression of cloned genes (e.g., virulence) or epitopes in mycobacteria and allows the rational construction of recombinant BCG polyvalent vaccines.—Authors' Summary

Jacobs, W. R., Jr., Snapper, S. B., Lugosi, L., Jekkel, A., Melton, R. E., Kieser, T. and Bloom, B. R. Development of genetic systems for the mycobacteria. Acta Leprol. 7 Suppl. 1 (1989) 203–207.

Requisite to a detailed understanding of the molecular basis of bacterial pathogenesis is a genetic system which allows for the transfer, mutation, and expression of specific genes. Genetic analysis of mycobacteria has been exceedingly difficult since the mycobacteria grow slowly and no natural efficient method of gene transfer within the pathogenic species has thus far been found. Using a molecular genetic approach, we have developed both the vectors and the methodology for efficient gene transfer in the mycobacteria. Initially, a novel type of mycobacteriophage vector was developed, termed a shuttle phasmid. This hybrid shuttle vector replicates in *Escherichia coli* as a plasmid and in mycobacteria as a phage, capable of introducing foreign DNA into a wide variety of mycobacterial species. A set of shuttle phasmids, constructed from a temperate mycobacteriophage, retained their ability to lysogenize their mycobacterial hosts and could thus introduce foreign DNA stably into mycobacterial cells. An *E. coli* gene conferring kanamycin resistance was cloned into these vectors and shown to express in the mycobacteria, thus providing the first selectable marker gene for subsequent genetic studies. Using kanamycin-resistance gene as a selection, the *Mycobacterium fortuitum* plasmid pAL5000 replicon, and electroporation, a plasmid transformation system has been developed for both *M. smegmatis* and BCG. We now plan to use these phage and plasmid systems to analyze, genetically, the virulence attributes of the pathogenic mycobacteria. In addition, by introducing and expressing foreign antigens in BCG, we hope to develop a novel recombinant multi-vaccine vehicle capable of conferring immunity to a variety of bacterial, viral, and parasitic pathogens.—Authors' Abstract

Katoch, V. M., Shivannavar, C. T. and Datta, A. K. Studies on ribosomal RNA genes of mycobacteria including *M. leprae*. Acta Leprol. 7 Suppl. 1 (1989) 231–233.

Information about specific genes, especially of pathogenic mycobacteria, could be used to unequivocally identify isolates of mycobacteria which are of clinical interest. Both eukaryotic and prokaryotic ribosomal RNA (rRNA) genes have been shown to comprise sequences which are conserved and others which are divergent. In the present study, rRNA genes from several cultivable mycobacteria including *Mycobacterium tuberculosis* and armadillo-derived *M. leprae* have been investigated. rRNA was isolated, made radioactive *in vitro* and then used to identify restriction fragments of DNA containing rRNA gene sequences. It was observed that restriction endonuclease patterns of rRNA genes are characteristic. By probing with homologous and heterologous rRNA probes, fragments hybridizing maximum with homologous probes could be identified, and it appears that sequences flanking the rRNA genes are not identical. These fragments need to be further sequenced to identify the nucleotide sequences specific to rRNA gene cluster. It would also be necessary to analyze several isolates of each species including armadillo-derived *M. leprae* before reaching any conclusions.—Authors' Summary

Lugosi, L., Jacobs, W. and Bloom, B. R. Transformation of BCG with plasmid DNA. Acta Leprol. 7 Suppl. 1 (1989) 256–257.

Pasteur 1173P2 and Japanese 172 BCG substrains were transformed with plasmid

pAL5000::plJ666 by electrophoration and assessed by the growth of kanamycin-resistant colonies. From the transformants pYUP plasmids were isolated containing plJ666 inserted into pAL5000. The introduction, persistence and the identity of the plasmid were monitored by reisolation from consecutive subcultures and restriction analysis. The effects of variables—age, viability, glycine pretreatment of BCG cultures, electroporation parameters—on transformation frequencies were analyzed. Consecutive transformation of BCG with plasmid DNA isolated from a BCG transformant increased the efficiency from the level of 10^1–10^2 obtained with the initial library to 10^3–10^4 colonies/μg DNA with the pYUB plasmids. Maintenance of plasmid and expression of kanamycin resistance in continuous subcultures were stable for 100 generations from the first successful transformation to the present. The introduction and stable expression of foreign DNA in BCG on a plasmid vector establishes a basis for the construction of polyvalent recombinant BCG vaccine vehicles expressing not only putative protective mycobacterial antigens, but also antigens for other infectious and malignant diseases.—Authors' Summary

Minnikin, D. E., Ridell, M., Wallerström, G., Besra, G. S., Parlett, J. H., Bolton, R. C. and Magnusson, M. Comparative studies of antigenic glycolipids of mycobacteria related to the leprosy bacillus. Acta Leprol. 7 Suppl. 1 (1989) 51–54.

The leprosy bacillus, *Mycobacterium leprae,* is a member of a small group of mycobacteria comprising the species *M. bovis, M. marinum, M. kansasii, M. tuberculosis, M. ulcerans* and related taxa. This relationship is based on the similarity of the characteristic lipid types in the cell envelope. *M. leprae* produces a phenolic glycolipid antigen which is species specific. This communication reports a comparison of the specificity of the lipid antigens of other members of this group of mycobacteria. *M. kansasii,* in accordance with previous studies, produces phenolic glycolipid and trehalose-based lipooligosaccharide antigens which do not crossreact with antisera raised against other mycobacteria. The phenolic glycolipid and an uncharacterized polar glycolipid, with the properties of a lipooligosaccharide, from *M. marinum* are also shown to be specific antigens. An acylated trehalose glycolipid antigen from *M. tuberculosis* H37Rv reacts strongly with antisera raised against the same strain and sera from 8 out of 10 tuberculosis patients. The phenolic glycolipid antigen, isolated only from *M. tuberculosis* "Canetti" variants, did not react with antisera raised against the type strain, *M. tuberculosis* H37Rv, although it had been shown previously to react with sera from tuberculosis patients. It is apparent that there are populations of the tubercle bacillus which differ in the lipid antigens expressed on their cell surface.—Authors' Summary

Nakamura, M. and Kohsaka, K. Effect of lyophilization on viability of *Mycobacterium leprae* multiplied in nude mouse. Acta Leprol. 7 Suppl. 1 (1989) 36–38.

In an effort to preserve *Mycobacterium leprae in vitro,* the effect of freezing and drying, i.e., lyophilization, on viability of *M. leprae* was studied. The viability of the bacilli was quantitatively measured with the foot-pad inoculation method using nude mice. The results obtained demonstrate that the viability of *M. leprae* was reduced approximately 10^{-2} to 10^{-3} from that of the starting material during the process of lyophilization; no viable bacilli were detected in the lyophilized sample containing $< 1.8 \times 10^3$ bacilli. On the other hand, the bacilli capable of multiplication in nude mouse foot pads were found in the lyophilized sample with $> 10^5$ bacilli. From the results obtained here, it could be suggested that there might be a possibility to preserve *M. leprae in vitro* by means of lyophilization.—Authors' Summary

Stackebrandt, E., Smida, J. and Kazda, J. The primary structure of the 16SrRNA of *Mycobacterium leprae*: its use in phylogeny and development of DNA probes. Acta Leprol. 7 Suppl. 1 (1989) 222–225.

Two long stretches of the 16S from *Mycobacterium leprae* were sequenced using reverse transcriptase and the chain termination technique. Homology values were calculated for 11 cultivable mycobacteria

and a phylogenetic tree constructed from evolutionary distance values (Knuc). Slow- and fast-growing mycobacteria used in this study form a taxonomic unit but were phylogenetically well separated. It could be confirmed that *M. leprae* is a true member of the slow-growing pathogenic mycobacteria branching off intermediate to other members of this subgroup. Comparison of the 16 rRNA primary structures reveals that the nucleotide sequence of *M. leprae* contains regions of sufficient variation to serve as potential target sites for DNA probes. Here we describe the designation of a DNA oligonucleotide and its use in dot blot hybridization experiments where it was directed against bulk RNA isolated from several mycobacteria.—Authors' Summary

Vishnevetsky, F. E., Yuschenko, A. A. and Anokhina, V. V. [Morphological characteristics of cell cultures from human and animal lepromas and the effect of antileprosy drugs on them.] Biull. Eksp. Biol. Med. **106** (1988) 737–741. (in Russian)

Leproma pieces obtained from leprosy patients and *Mycobacterium leprae* infected animals were cultivated by the method of primary explantation. It is noted that the development of a monolayer from macrophages overloaded with *M. leprae* is a characteristic common to the lepromas of various origin. Antileprosy activity of the drugs under study was assessed by the rate of decrease in mycobacterial load of macrophages. Species features of cultivated lepromas from man, nine-banded armadillo and mouse are characterized. While cultivating lepromas from leprosy patients the peculiarities of tissue culture organization are found, representing immune status and prognosis of specific therapy.—Authors' English Abstract

Wheeler, P. R. Pyrimidine biosynthesis in *Mycobacterium leprae* and other intracellular mycobacteria. Acta Leprol. **7** Suppl. 1 (1989) 33–35.

All the enzymes which could be detected in mycobacteria that are unique to the *de novo* pathway of pyrimidine biosynthesis were shown (in cell extracts) in *Mycobacterium leprae.* Activity of the first enzyme in the pathway, aspartate transcarbamylase, was shown in *M. microti* and *M. avium* grown *in vivo* and in liquid Dubos medium. This suggested that the very low activity of pyrimidine biosynthesis *de novo* in intact *M. microti* and *M. avium* grown *in vivo,* and the failure to detect any activity in *M. leprae,* is a result of feedback inhibition of the metabolic pathway for pyrimidine biosynthesis as opposed to any lack of metabolic capability.—Author's Summary

Williams, D. L. and Gillis, T. P. A study of the relatedness of *Mycobacterium leprae* isolates using restriction fragment length polymorphism analysis. Acta Leprol. **7** Suppl. 1 (1989) 226–230.

The inability to cultivate *Mycobacterium leprae in vitro* has severely hampered comprehensive phenotypic analysis of individual isolates, leaving unanswered the question of the relatedness of these isolates. Since the nucleotide sequence of a bacterial chromosome is its "genetic fingerprint," we have employed the use of restriction fragment length polymorphism (RFLP) analysis of chromosomal DNA of *M. leprae* isolates to assess the relatedness among these isolates. DNA of *M. leprae* was harvested from infected armadillo tissue originally inoculated with bacilli from lepromatous lesions of human patients from geographically distinct regions of the world. Restriction endonuclease (*Eco*RI, *Pst*I, and *Pvu*I) digests of chromosomal DNA were analyzed using DNA probes encoding all or part of the 28-kDa, 65-kDa and 70-kDa proteins of *M. leprae.* Comparison of the resultant autoradiographs showed that the RFLP patterns were all identical, indicating that these isolates contained no polymorphism with respect to the restriction endonuclease sites analyzed. These results indicated that the *M. leprae* isolates tested in this study were indistinguishable at the genotypic level, suggesting the possibility of homogeneity among members of the species *M. leprae.*—Authors' Abstract

Experimental Infections

Srinivasan, R. and Rao, P. R. Humoral immune responses in dapsone treated *M. leprae* infected mice. Indian J. Lepr. **61** (1989) 151–159.

Humoral responses to sheep erythrocytes in *Mycobacterium leprae* infected mice were studied through 52 weeks and were found to be directly related to the bacterial load. However, treatment with dapsone (DDS) in the last 12 weeks of infection resulted in an initial enhancement of the humoral responses followed by a gradual decrease, although they were still significantly higher at the end of the study.—Authors' Abstract

Vishnevetsky, F. E., Yushin, M. Y., Ayupova, A. K. and Urlyapova, N. G. [Reproduction of experimental leprosy infection in mice with previously induced insufficency of mononuclear phagocyte system.] Biull. Eksp. Biol. Med. **106** (1988) 574–578. (in Russian)

CBA mice with induced insufficiency of monoclear phagocyte system (MNPS) were inoculated into the foot pad with *Mycobacterium leprae* in a dose of 1×10^4. A significantly accelerated multiplication of *M. leprae* was noted in the sites of inoculation with the development of a generalized infectious process and appearance 6 months after inoculation of lepromatous structures in spleen, liver and other internal organs as compared with the animals with unchanged MNPS. The data obtained suggest that insufficiency in macrophage component of cell-mediated immunity might underline the susceptibility to leprosy infection.—Authors' English Summary

Epidemiology and Prevention

Dewapura, D. R. The current state of leprosy control activities in Sri Lanka. Lepr. Rev. **60** (1989) 39–44.

In Sri Lanka the overall prevalence of leprosy was 0.14 per 1000 population and the incidence 0.07 per 1000 population at the end of 1987. Although the endemicity is low in the island, interruption of disease transmission has not yet been achieved as the annual detection of new cases and the child rate have been gradually rising. The major activities of the leprosy control program are case-finding, treatment and defaulter retrieval, health education, rehabilitation and training. The field program is implemented through 15 specially trained paramedical workers. In addition there are 5 medical officers attached to the Anti-leprosy Campaign. The Director of the Anti-leprosy Campaign is in overall charge of the National Leprosy Programme and is also project manager for the Sri Lanka Emmaus Leprosy Control Project.—Author's Summary

Naik, S. S., Ganapati, R., Shah, D. H., Dandekar, S. R. and Sahasrabudhe, R. V. Identification of "high risk group" of leprosy in children using serological and lepromin test: a preliminary report. Indian J. Lepr. **61** (1989) 173–175.

The presence of specific antigen of *Mycobacterium leprae* or its antibodies in biological fluids of any individuals indicates the exposure of *M. leprae* infection, and negative reaction with lepromin suggests the immunological incompetence of the individual with *M. leprae*. The existence of both together therefore suggests the individual to be at a "high risk" and he/she perhaps may develop clinical leprosy in near future. Keeping this in mind, sera samples of 174 children (age 10–14 years) residing in the leprosy colony and 75 children of an orphanage located in the same area and in same age group were evaluated for the presence of antibodies to PGL-1 and circulating immune complex and *in vivo* lepromin re-

action. For the lepromin reaction armadillo-derived antigen was used. The clinical examination at zero day of these children was carried out; all of them had no evidence of leprosy. The bacteriological examination for the presence of *M. leprae* was done from the skin of an elbow and one of the ear lobes of students who gave a negative result of late lepromin reactivity. All these smears were reported negative for the presence of *M. leprae* which has ruled out already existing leprosy among them. The children from the leprosy colony were followed up for 2 years by clinical examination, and the children in the orphanage were taken up recently and could be followed up for 6 months only.—(*From* the Article)

Rajagopalan, M. S., Balakrishnan, S. and Ramu, G. Subclinical infection and the relative risk of developing leprosy: a statistical approach. Indian J. Lepr. **61** (1989) 169–172.

Subclinical infection in contacts of leprosy patients was identified by the FLA-ABS test and the serum antibody competition test (SACT). The risk of developing leprosy and the confidence intervals were worked out. The importance of expressing the risk ratio and confidence interval of the tests is brought out. This method is a useful adjunct to the routine statistical methods in epidemiological studies.—Authors' Abstract

Rao, P. S., Kumaravelu, P., Muthiah, B. and Durgambal, K. A study into bacteriological positivity and treatment of beggar leprosy patients. Indian J. Lepr. **61** (1989) 216–221.

One hundred beggar leprosy patients were medically examined and skin smears were taken for bacteriological positivity for AFB. Information regarding their treatment was collected. Twenty percent of them were found bacteriologically positive and 40% of the positive cases were not taking treatment. Epidemiological and operational implications of the findings are discussed.—Authors' Abstract

Saylan, T., Sutlas, M., Yuksel, A., Cakiner, T. and Aytekin, H. The characteristics and mode of detection of the new patients encountered in the leprosy endemic province of Van within the last five years. Indian J. Lepr. **61** (1989) 225–228.

Between 1983–1987, the Istanbul Leprosy Centre (ILC) organized in Van a leprosy education program for medical personnel and the local population. Subsequently whole population surveys and case contact surveys were carried out independently in different regions. Sixty-six new cases were detected during those years; 56 (85%) patients were diagnosed by ILC teams at the field and at the hospital. In 49 (74%) of the 66 there was one or more close contact within the family. In 17 (26%) there was none, but there were old patients in the village or nearby. It is concluded that the education of the local medical authorities and the population is of utmost importance for the early diagnosis and patient close contact surveys are the best for our country.—Authors' Abstract

Rehabilitation

Gonzalez del Cerro, S. M. A. [The stigma of leprosy.] Rev. Argent. Dermatol. **70** (1989) 36–43. (in Spanish)

The objective of this multidisciplinary work is the study of the leprosy patient's stigma and his psychological and social connotations. Its main fundament is Goffman's "Stigma." The method has consisted in going from the general to the particular or, in other words, from the study of the stigma in general to the study of the stigma in leprosy, giving concrete examples. The following results were obtained. The stigma of leprosy has four components: physical, psychological, moral and social. The patient

does not conform with the patterns of identity imposed by society and is rejected. The patients form a characteristic endogroup. Leprosy is an interference in the communication with the rest. The stigma is contagious. Perceptibility is one fundamental characteristic of the hansenian. The patient runs a "moral race" in which the internment in a specific establishment has notable influence. The management of the information of the same is hidden, using control technics for the information. The patients cover ambivalencies with respect to himself and his group. The conclusion of this investigation helps to completely face the treatment of the hansenian for it bears in mind his somatic, mental and social aspects.—Author's English Summary

Other Mycobacterial Diseases and Related Entities

Batra, P. P., Takeda, K. and Kreye, W. Studies on adenylate kinase (ATP:AMP phosphotransferase) of *Mycobacterium marinum* (ATCC 927). Acta Leprol. 7 Suppl. 1 (1989) 25–29.

The enzyme adenylate kinase (ATP:AMP phosphotransferase) was purified from *Mycobacterium marinum* as described previously with an additional step involving affinity chromatography on Cibacron blue. The molecular weight of the final enzyme preparation was estimated to be 22,500 on polyacrylamide-gel electrophoresis under denaturing conditions. The preliminary amino acid analysis indicated the presence of two histidine residues. Photooxidation in the presence of methylene blue caused complete inactivation of the enzyme, but the loss of activity could be prevented by the addition of ATP, AMP or adenosine-(5')-pentaphospho-(5')-adenosine, indicating that at least one histidine residue is involved at the active site. A circular dichroic study indicated that the enzyme consists of 24% α-helix, 30% β-structure, and 46% random coil. The bacterial cells induced with antimycin A and light (particularly with the former) appeared to have somewhat increased adenylate kinase activity, although the Km and Vmax values were unchanged.—Authors' Summary

Blaauwgeers, J. L. G., Das, P. K., Slob, A. W. and Houthoff, H. J. Human gut wall reactivity to monoclonal antibodies against *M. avium* glycolipid in relation to Crohn's disease (preliminary results). Acta Leprol. 7 Suppl. 1 (1989) 138–140.

In order to evaluate the role of mycobacteria in the pathogenesis of Crohn's disease (CD), four monoclonal antibodies (MAbs) raised against *Mycobacterium avium*-specific glycolipid were tested on bowel resection specimens of CD patients and relevant controls. Two of the MAbs had shown a positive reaction to a CD-mycobacteria isolate (CD-Myc). The same two MAbs showed a positive reaction within the gut wall, not only in CD patients, but also in the controls. In non-CD controls however, the positive cells were limited to the lamina propria, while in CD patients positivity was also found in the submucosa and subserosa. Furthermore, the mean number of positive cells in CD patients tends to be higher than in the control groups. Using double-staining techniques the positive cells appeared to be B cells of the IgA isotype. These preliminary results indicate that mycobacteria might play a role in the pathogenesis of Crohn's disease.—Authors' Summary

Blom-Potar, M.-C., David, H. L. and Rastogi, N. Isoenzyme profiles in slowly growing and difficult-to-grow mycobacteria. Acta Leprol. 7 Suppl. 1 (1989) 48–49.

The cell-free extracts of 13 slow-growing mycobacteria were run on polyacrilamide gels (PAGE) and the various protein bands obtained were tested for peroxidase and catalase enzyme activities. The results obtained were compared to those obtained with *Mycobacterium fortuitum* and *M. chelonae.* Based only on a limited number of strains employed, it is suggested that these isoen-

zyme patterns may permit a better separation of "Wood-pigeon" mycobacteria from both *M. avium* and *M. paratuberculosis* and also give distinct profiles for other species used. These results further suggest the potential of isoenzymes as taxonomic markers.—Authors' Summary

Blom-Potar, M.-C., David, H. L. and Rastogi, N. Isoenzymes as tools to discriminate various subdivisions in the *Mycobacterium fortuitum* complex. Acta Leprol. **7** Suppl. 1 (1989) 39–43.

Methods for the characterization of catalase, peroxidase, β-glucosidase, esterase, and β-lactamase mycobacterial isoenzymes are described. These methods were applied to examine strains of the *Mycobacterium fortuitum* complex. *M. fortuitum, M. peregrinum, M. chelonae-M. abscessus* and an unnamed species had distinct isoenzyme profiles. *M. chelonae* and *M. abscessus* could not be satisfactorily differentiated using the described methods.—Authors' Summary

Boddingius, J. and Dijkman, H. P. Immunogold labeling method for *Mycobacterium leprae*-specific phenolic glycolipid in glutaraldehyde–osmium-fixed and araldite-embedded leprosy lesions. J. Histochem. Cytochem. **37** (1989) 455–462.

Phenolic glycolipid (PGL)-I, a *Mycobacterium leprae*-specific antigen currently used for serodiagnosis of preclinical leprosy, has thus far not been localized subcellularly in leprosy bacilli and their host cells. In this study, we developed an immunogold-labeling technique for qualitative identification of PGL-I sites in glutaraldehyde-osmium-fixed and araldite-embedded *M. leprae* and host macrophages in human skin biopsies. Such "hard-fixed," plastic-embedded skin and nerve biopsies from patients with varying cell-mediated immunity to leprosy are amply available worldwide. Our method involves etching of plastic sections with H_2O_2, incubation with swine serum to eliminate nonspecific labeling, and long (22 hr) incubation at room temperature with monoclonal antibodies to PGL-I. Gold labeling was seen predominantly on the cell walls of *M. leprae,* in vacuolar spaces of bacillated phagolysosomes, and occasionally on the cytoplasm and cell membrane of *M. leprae.* Host macrophage cytoplasm was labeled very infrequently. This technique allows studies on possibly persisting antigenic PGL-I in multibacillary leprosy patients during or after multidrug therapy. The method may also prove useful for subcellular localization of specific bacterial lipids in other mycobacterial diseases, including tuberculosis.—Authors' Abstract

Brown, A. R., Rieder, J. T. and Webster, H. K. Prolonged elevations of soluble interleukin-2 receptors in tuberculosis. Am. Rev. Respir. Dis. **139** (1989) 1036–1038.

In order to determine whether tuberculosis is associated with elevated levels of soluble interleukin-2 receptors (SIL-2R), patients with both pulmonary (N = 12) and extrapulmonary (N = 8) disease were studied. SIL-2R were measured in sera using an enzyme-linked immunosorbent assay (ELISA). Prior to treatment, all 20 patients had levels of SIL-2R significantly greater than those of the 14 control subjects. Longitudinal study of patients with pulmonary disease revealed that levels of SIL-2R remained elevated in 11 of 12 patients after 2 months, and in 4 of 6 patients after 3 months of treatment. Those findings suggest that tuberculosis is characterized by prolonged activation of the immune system despite optimal chemotherapy and that SIL-2R may distinguish active immunity from immunologic memory to this infection.—Authors' Summary

Cruaud, P., Papa, F., David, H. L. and Daffé, M. Specificity and antigenicity of mycoside G and other glycolipids from *Mycobacterium marinum.* Acta Leprol. **7** Suppl. 1 (1989) 94–97.

The purpose of this work was to examine the immunologic properties of the phenolic glycolipid produced by *Mycobacterium marinum* (mycoside G). Cell mass processed with organic solvents yielded a lipid extract containing mycoside G and four other glycolipid fractions (G2 to G5). No mycoside G was found in the *M. marinum*-type strain. Neither mycoside G, nor fractions G2, G3 and G4 were immunogenic in

the rabbit; whereas fraction G5 was very immunogenic. The immune serum raised in the rabbit showed that fraction G5 is species specific since it was consistently detected in all *M. marinum* strains examined so far.—Authors' Summary

Das, P. K., Soolingen, D., Spies, J. and Chand, A. (in collaboration with Ottenhoff, T. H. M.). Concentration dependent functional responsiveness to subcellular antigenic extracts of BCG and different mycobacterial PPDs, of tuberculin (Mtb-PPD) reactive human T-cell lines. Acta Leprol. 7 Suppl. 1 (1989) 164–168.

Mycobacterium tuberculosis purified protein derivative (Mtb-PPD) as tuberculin, other mycobacterial PPD-preparations and subcellular antigenic extracts of BCG showed varying concentration dependent bimodal effects in lymphoproliferative assay (LA) of Mtb-PPD generated human CD4+ T-cell lines (TCLS). Inhibitory effect on LA by high dose of Mtb-PPD is correlated with the inhibition of IL-2 production during the antigen-induced stimulation. Consequently, maximal and inhibitory concentrations of different antigens in dose response LA varied for different TCLS. However, the inhibitory effect can be overcome by the high concentration of noninhibitory antigen for the particular TCL. These results indicate that a) dose-dependent LA is essential for the evaluation of antigen specificity of TCL; b) some mycobacterial antigen may induce anergy to certain concentration whereas some others exert positive effect to overcome such anergy related to inhibition or production of IL-2.—Authors' Summary

David, H. L., Clavel-Sérès, S., Clément, F., Lazlo, A. and Rastogi, N. Methionine as methyl-group donor in the synthesis of *Mycobacterium avium* envelope lipids, and its inhibition by DL-ethionine, D-norleucine and DL-norleucine. Acta Leprol. 7 Suppl. 1 (1989) 77–80.

The radioactivity from ^{3}H-methyl methionine was rapidly incorporated into the surface lipids of *Mycobacterium avium*. The transmethylation reaction was efficiently inhibited by DL-methionine, D-norleucine and DL-norleucine. The structure of the outlayer of the *M. avium* envelope was profoundly altered in the bacteria treated with DL-norleucine.—Authors' Summary

David, H. L., Thorel, M. F., Fréhel, C. and Rastogi, N. Serologic and immunocytochemical analysis of the *Mycobacterium avium* cell envelope. Acta Leprol. 7 Suppl. 1 (1989) 55–58.

Whole cell sonicates of *Mycobacterium avium* ATCC 15769 and subcellular fractions (CYT, cytosol; CM, cytoplasmic membrane; CWS, delipidated cell wall skeleton; OL, native lipids; and SDS, sodium dodecylsulfate extract of whole cells) were injected into rabbits to produce corresponding antisera. Immunoelectrophoretic analysis and immunochemical observations using electron microscopy showed that few of the antigens synthesized intracellularly were exported and located in the bacterial outer layers, and that the outer layers contained wall specific antigens possibly *in situ* assembled.—Authors' Summary

de Chastellier, C. and Lang, T. Bacterial antigen processing in macrophages infected with the obligate intracellular bacterium, *Mycobacterium avium*. Acta Leprol. 7 Suppl. 1 (1989) 175–176.

The intracellular pathway followed by *Mycobacterium avium* antigens was studied by immunofluorescence and immunoelectron microscopy after infection in bone-marrow-derived macrophages with live or gamma-ray-killed bacteria. Rabbit antiserum raised against whole sonicated bacteria was used to localize antigens. The acidity of the phagosomal compartment was also investigated by immunoelectron microscopy. Preliminary results seem to indicate the phagosomal compartment is less acidified than lysomes and that *M. avium* antigens are very slowly processed.—Authors' Summary

Fattorini, L., Fiorentino, D., Amicosante, G., Franceschini, N., Oratore, A. and Orefici, G. Beta-lactamase production and biological characteristics in nitrosoguanidine induced *Mycobacterium fortuitum* mu-

tants. Acta Leprol. 7 Suppl. 1 (1989) 44–47.

In order to elucidate the role of β-lactamase in the resistance of *Mycobacterium fortuitum* to β-lactams, *M. fortuitum* ATCC 19542 and three mutants, strains D 316, D 319 and D 170 obtained from it by nitrosoguanidine treatment, were studied. Furthermore the kinetics of the β-lactamase production during the bacterial growth and many biochemical and enzymatic characteristics of parent and mutant strains were investigated. Amoxicillin MICs well correlated with the β-lactamase production in the high producer strains D316 and D 319; on the other hand, in strain D 170 a high MIC was joined with a moderate production of the enzyme showing that not only β-lactamase but also other mechanisms can be effective in the resistance of *M. fortuitum* to β-lactams. Clavulanic acid, an inhibitor of β-lactamase, reduced MICs to amoxicillin in high and in low producer strains. The production of extracellular β-lactamase occurred in a *M. fortuitum* mutant strain mainly during the stationary phase, indicating that a cell wall damage or initial autolysis could be responsible for the release of enzyme. Enzymatic and biochemical characteristics were not affected by nitrosoguanidine treatment except for nitrate test which showed only a weak positivity in high-producer strains.—Authors' Summary

Frehel, C. and Rastogi, N. Phagosome-lysosome fusions in macrophages infected with *Mycobacterium avium*: role of mycosides-C and other cell surface components. Acta Leprol. 7 Suppl. 1 (1989) 173–174.

The phagosome-lysosome fusions (PLF) were assessed in case of bone-marrow macrophages infected by the opportunistic species *Mycobacterium avium,* employing the acid-phosphatase (AcPase) electron-cytochemistry. The role of surface components was evaluated by coating the bacteria prior to phagocytosis by specific *M. avium* antiserum or the anti-mycosides-C serum raised in rabbits. PLF was evaluated under the electron microscope during (2, 4 hr), or after (24 hr) phagocytosis. The preliminary results suggest that although *M. avium* surface components intervene in PLF inhibition, the role of mycosides-C among these surface components (effectively intervening in PLF inhibition) is questionable.—Authors' Summary

Frehel, C., Thorel, M.-F. and Rastogi, N. Evidence that host-recycling of *Mycobacterium avium* preserves its ability to hinder macrophage killing functions. Acta Leprol 7 Suppl. 1 (1989) 160–163.

The opportunistic pathogen *Mycobacterium avium* was selected as a model for the study of the bacterial cell envelope and resistance to macrophage-killing functions. We hereby demonstrate that characteristic features of *M. avium,* e.g., existence of a polysaccharide-rich outer layer (POL), presence of a protective capsule also called electron-transparent zone around phagocytized bacteria, and inhibition of phagosome-lysosome fusions, were better expressed by bacteria recently isolated from infected rabbits than by bacteria subcultured in laboratory media. Our data appeared to confirm previous suggestions that *M. avium* regulatory mechanisms are such that during laboratory growth of these bacteria, synthesis of surface components which may be important concerning their virulence properties is effectively diminished.—Authors' Summary

Gorzynski, E. A., Gutman, S. I. and Allen, W. Comparative antimycobacterial activities of difloxacin, temafloxacin, enoxacin, pefloxacin, reference fluoroquinolones, and a new macrolide, clarithromycin. Antimicrob. Agents Chemother. **33** (1989) 591–592.

The activities of fluoroquinolones and a new macrolide against 30 clinical isolates of *Mycobacterium tuberculosis* were determined *in vitro* by agar diffusion. In order of relative potencies against *M. tuberculosis,* temafloxacin (MIC for 90% of isolates [MIC_{90}], 2.3 μg/ml) was at least as active as the reference quinolones ofloxacin (MIC_{90}, 2.4 μg/ml) and ciprofloxacin (MIC_{90}, 4.3 μg/ml). Less active were difloxacin (MIC_{90}, 4.7 μg/ml), pefloxacin (MIC_{90}, 6.7 μg/ml), and enoxacin (MIC_{90}, 8.3 μg/ml). The macrolide clarithromycin was more potent than eryth-

romycin but less potent than the fluoroquinolones. Our results suggest that the newer fluoroquinolones and clarithromycin should be included with ciprofloxacin and ofloxacin in pharamcokinetic studies that may lead to trials in human subjects with mycobacterial infections.—Authors' Abstract

Green, E. P., Moss, M. T., Hermon-Taylor, J. and McFadden, J. J. Insertion elements in mycobacteria. Acta Leprol. 7 Suppl. 1 (1989) 239–242.

We have isolated and characterized a repetitive element from the genome of *Mycobacterium paratuberculosis.* This repetitive element shows many features characteristic of a bacterial insertion element.—Authors' Summary

Han, Y., He, H., Oka, S. and Yano, I. Relationships between structure and biological activity of the mycolic acid-containing glycolipids from *Nocardia asteroides* "sensu stricto" and related species. Acta Leprol. 7 Suppl. 1 (1989) 130–132.

To reveal the taxonomical situation of *Nocardia asteroides* "sensu stricto," we compared the mycolic acid and mycolic acid-containing glycolipid composition and their granulomagenic activies in mice. The major glycolids were glucose mono- and dimycolate, trehalose mono- and dimycolate, and several unknown glycolipids, commonly, although the relative amount differed from strain to strain. On the other hand, molecular species composition of mycolic acids differed distinctively among the three closely related species: *N. asteroides* "sensu strico," *N. farcinica* and *N. nova.* GC/MS analysis showed the most abundant species of mycolic acids were $C_{50(52)}$ in *N. asteroides,* $C_{54(52)}$ in *N. farcinica* and $C_{58(56)}$ in *N. nova,* respectively, with a different α-alkyl branch. Glucose mycolate and trehalose dimycolate possessing C_{50} mycolic acid showed a strong activity for granuloma formation in mice.—Authors' Summary

Hoffner, S. E., Källenius, G., Beezer, A. E. and Svenson, S. B. Studies on the mechanisms of the synergistic effects of ethambutol and other antibacterial drugs on *Mycobacterium avium* complex. Acta Leprol. 7 Suppl. 1 (1989) 195–199.

Synergistic effects of combinations of antimycobacterial drugs on *Mycobacterium avium* complex (MAC) *in vitro* were studied by radiometric respirometry. Pronounced synergy was seen for several drug combinations where ethambutol was found to be the key drug in the synergistic potentiation. Microcalorimetric studies show that a very rapid physico-chemical interaction occurs between the cell surface of MAC and ethambutol. When MAC cells were pretreated with ethambutol and then subjected to streptomycin, the thermal response significantly differed from that seen with MAC cells which had not been pretreated. The typical thermal effects of the interaction of ethambutol with live and UV-killed MAC cells were not seen with heat-killed MAC cells. It is proposed that specific cell-surface protein(s) act as receptors in the initial interaction with ethambutol.—Authors' Summary

Ikawa, H., Kaneda, K., Goto, K., Tsuyuguti, I., Ueno, Y., Hua, H., Oka, S. and Yano, I. Rapid and precise diagnosis of atypical mycobacterial infection by chemotaxonomical and immunological methods. Acta Leprol. 7 Suppl. 1 (1989) 85–88.

The species of 205 strains of acid-fast bacteria isolated from swine and human mycobacteriosis were identified chemotaxonomically and numericaltaxonomically. The species of the isolates which were identified numericaltaxonomically as *Mycobacterium avium intracellulare* (MAI) complex were further classified by using both thin-layer chromatography of the antigenic glycopeptidolipids (GPL) from the bacteria and seroagglutination test devised by Schaefer. These MAI complex from swine fell into serotype 8 (45 strains), serotype 4 (32 strains), serotype 9 (9 strains) and untypable (9 strains), respectively. In contrast to swine, human isolates covered more wide ranges of serotypes such as serovar 7, 12, 16 besides serovar 4, 8 and 9. Furthermore, enzyme-linked immunosorbent assay (ELISA) which is based on the type specific glycolipid antigen and infected swine/human sera was applied to distinguish serological variants of the MAI

complex. Of the 14 cases in swine and five in human that had been typed by both the seroagglutination reaction and the thin-layer chromatography (TLC) the 13 in swine and two in human cases showed clear coincidence with the results of ELISA. The results demonstrated that ELISA using infected sera was especially useful, and it was recommended from the sensitivity and rapidity as an adjunct to seroagglutination test and thin-layer chromatography for the identification of serotypes of MAI complex.—Authors' Summary

Labidi, A. and Thoen, C. O. Genetic relatedness among *Mycobacterium paratuberculosis* and *M. avium* complex. Acta Leprol. 7 Suppl. 1 (1989) 245–248.

Total DNA was extracted from *Mycobacterium paratuberculosis* (ATCC 19698) and from *M. avium* complex (ATCC 25291) cultivated on RVB-10-enriched liquid media. Restriction endonuclease analysis of total DNA was performed with 34 enzymes, and DNA digestion profiles were compared. Fifteen enzymes revealed important differences between the two species. Two pairs of enzymes (*Eco*RII, *Bst*NI) and (*Mbo*1, *Sau*3AI) provide evidence for the presence of *dcm*I and *dam* methylation in DNA of *M. avium* complex and *M. paratuberculosis.* The differences in DNA fragments of these two species could be of potential value in differentiating these clinically significant mycobacteria.—Authors' Summary

Labidi, A. and Thoen, C. O. Genetic relatedness among *Mycobacterium tuberculosis* and *M. bovis.* Acta Leprol. 7 Suppl. 1 (1989) 217–221.

Total DNA from two slow-growing pathogenic mycobacterial species propagated *in vitro* was isolated, digested with each of 34 restriction endonucleases, and analyzed by agarose gel electrophoresis. The most resolved patterns for *Mycobacterium tuberculosis* (ATCC 27294) and for *M. bovis* (ATCC 19210) were obtained respectively using (*Bam* HI, *Dra*I, *Cla*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I and *Xma*I). The patterns produced for these strains were reproducible and distinguishable from each other respectively using (*Hin*dIII, *Dra*I, *Eco*RI, *Mbo*I, *Sau*3AI and *Ava*I). However, with several enzymes (*Sal*I, *Asu*I, *Sau*96I, *Msp*I and *Hpa*II) the patterns for *M. tuberculosis* and *M. bovis* were similar. Evidence was obtained for the presence of *dam* and *dcmI* methylations in the DNA of each mycobacterial species respectively using (*Mbo*I, *Sau*3AI, *Eco*RII, *Bst*NI, *Sau*96I and *Asu*I).—Authors' Summary

Lazraq, R., Moniz-Pereira, J., Clavel-Sérès, S., Clément, F. and David, H. L. Restriction map of mycobacteriophage D29 and its deletion mutant F5. Acta Leprol. 7 Suppl. 1 (1989) 234–238.

A physical map of mycobacteriophage D29 was constructed, including positions for 25 restriction sites for 9 endonucleasic enzymes. D29 DNA contains about 48 150 bp. Analysis of a deletion mutant (F5) has allowed us to determine the location of two nonessential regions in the genome, allowing further insertion of foreign genes and construction of cosmids.—Authors' Summary

Luquin, M., Lopez, F. and Ausina, V. Capillary gas chromatographic analysis of mycolic acid cleavage products, cellular fatty acids, and alcohols of *Mycobacterium xenopi.* J. Clin. Microbiol. **27** (1989) 1403–1406.

The fatty acids, alcohols, and mycolic acids of 26 strains of *Mycobacterium xenopi* were studied by capillary gas chromatography and thin-layer chromatography. All strains contained α-, keto-, and ω-carboxymycolates. The primary mycolic acid cleavage product was hexacosanoic acid. The fatty acid patterns and, especially, the presence of 2-ducosanol are characteristic markers of *M. xenopi.*—Authors' Abstract

Martín-Casabona, N., Gonzalez Fuente, T., Arcalis Arce, L., Otal Entraigas, J. and Vidal Pla, R. Evaluation of a phenolglycolipid antigen (PGL-Tb 1) from *M. tuberculosis* in the serodiagnosis of tuberculosis: comparison with PPD antigen. Acta Leprol. 7 Suppl. 1 (1989) 89–93.

Sera from 38 tuberculous patients and 62 healthy controls (31 PPD skin-test positive

and 31 negative) were assayed, by enzyme-linked immunosorbent assay (ELISA), to test the activity of IgG and IgM antibodies against purified protein derivative (PPD) antigen and a phenolglycolipid antigen (PLG-Tb 1) isolated and purified from *Mycobacterium tuberculosis* strain Canetti. Using PPD antigen, the sensitivity and specificity were, respectively, 50% and 93.5% for IgG and 71.1% and 59.7% for IgM antibody activity. Against PGL-Tb 1 antigen, IgG had a sensitivity of 94.7% and the specificity was 96.8%; for IgM antibody they were 65.8% and 75.8%, respectively. The ELISA using PGL-Tb 1 antigen could be a useful way to develop a rapid technique to aid in the diagnosis of tuberculosis.—Authors' Summary

Mukhopadhyay, A., Chaudhuri, G., Arora, S. K., Sehgal, S. and Basu, S. K. Receptor-mediated drug delivery to macrophages in chemotherapy of leishmaniasis. Science **244** (1989) 705–707.

Methotrexate coupled to maleylated bovine serum albumin was taken up efficiently through the "scavenger" receptors present on macrophages and led to selective killing of intracellular *Leishmania mexicana amazonensis* amastigotes in cultured hamster peritoneal macrophages. The drug conjugate was nearly 100 times as effective as free methotrexate in eliminating the intracellular parasites. Furthermore, in a model of experimental cutaneous leishmaniasis in hamsters, the drug conjugate brought about more than 90% reduction in the size of foot pad lesions within 11 days. In contrast, the free drug at a similar concentration did not significantly affect lesion size. These studies demonstrate the potential of receptor-mediated drug delivery in the therapy of macrophage-associated diseases.—Authors' Abstract

Nikolayan, L. T., Karapetyan, E. T. and Pospelov, L. E. [Classes I and II antigens and levels of IgG antibodies in patients with infiltrative tuberculosis of the lungs.] Probl. Tuberk. **3** (1989) 49–51. (in Russian)

Sixty patients with infiltrative tuberculosis of the lungs showing different dynamics of the process were examined. In the patients with unfavorable process of the disease antigens A2, B13, B16 and DR2 were more frequent than in the patients with favorable process of the disease in whom antigens A9, B12, C_w4 and DR5 were more frequent. It suggested that the presence of antigen DR2 was a certain risk factor as to development of tuberculosis and its presence in tuberculous patients could indicate unfavorable outcome of the disease. The levels of the specific antituberculous antibodies to PPD were also indicative of favorable and unfavorable processes of pulmonary tuberculosis.—Authors' English Summary

Papa, F., Laszlo, A., David, H. L. and Daffé, M. Serological specificity of *Mycobacterium tuberculosis* glycolipids. Acta Leprol. 7 Suppl. 1 (1989) 98–101.

Six glycolipid fractions can be extracted from lipid crude extracts of Canetti type strains of *Mycobacterium tuberculosis*. Among these fractions are two phenolglycolipids. The major component is a triglycosyl phenolphthiocerol dimycocerosate (PGL-Tb 1) and the second is a monoglycosyl diacyl phenolphthiocerol identical to mycoside B of *M. bovis*. Similar glycolipid compounds can also be found in wild strains of *M. tuberculosis* recently isolated from tuberculous patients. One of these compounds has been identified as PGL-Tb 1.—Authors' Summary

Perkins, J. D., Rakela, J., Sterioff, S., Banks, P. M., Wienser, R. H. and Krom, R. A. F. Immunohistologic pattern of the portal T-lymphocyte infiltration in hepatic allograft rejection. Mayo Clin. Proc. **64** (1989) 565–569.

Monoclonal antibodies were used to identify helper T cells (T_H) and suppressor/cytotoxic T cells ($T_{S/C}$) in liver allograft biopsy specimens obtained 7, 21, 90, 180, and 365 days postoperatively and then annually or during episodes of graft dysfunction and after treatment of rejection episodes. Biopsy specimens were obtained from 70 hepatic allografts from patients treated with cyclosporine and corticosteroids. Rejection was diagnosed by the presence of appropriate

laboratory and light microscopic findings and at least 16 weeks of follow up to exclude other causes of graft dysfunction. Three immunohistologic patterns were noted: no or only a trace of T lymphocytes, predominantly T_H infiltrate with or without a small amount of $T_{S/C}$ cells (portal T_H), and a mixture of T_H with an equal or greater number of $T_{S/C}$ infiltrate (portal mix). Of 68 biopsy specimens obtained during quiescent periods, only 3 had a portal tract T-lymphocyte infiltrate. Of 30 protocol biopsy specimens, 24 contained such an infiltrate a mean of 12.4 days before biochemical and routine histologic indications of rejection in the allograft. At the time of the rejection episode, 33 biopsy specimens were immunohistologically labeled; portal tract T-lymphocyte infiltrate was predominantly T_H in 8 and a mixture of T_H and $T_{S/C}$ in 25. All rejection episodes with a predominantly T_H pattern responded to methylprednisolone. Of the 25 rejection episodes with a portal mix pattern, only 3 responded to methylprednisolone. Eighty-seven biopsy specimens were obtained more than 10 days after treatment of rejection. Of 31 specimens obtained after resolution of rejection, 25 had no cells in the portal tract, and 43 of 56 biopsy specimens contained either portal T_H or portal mix pattern when the rejection episodes were persistent. Thus, the presence of portal T_H or portal mix pattern in biopsy specimens from hepatic allografts may be an early sign of immunologic rejection. By determining the T-lymphocyte pattern in hepatic allograft biopsy specimens during rejection, the proper course of antirejection therapy may be predicted. A portal T-lymphocyte pattern persists in hepatic allograft rejection episodes that do not resolve.—Authors' Abstract

Pimsler, M., Sponsler, T. A. and Meyers, W. M. Immunosuppressive properties of the soluble toxin from *Mycobacterium ulcerans*. J. Infect. Dis. **157** (1988) 577–580.

Buruli ulcer (caused by *Mycobacterium ulcerans*) is the product of a soluble necrotizing toxin from the infecting organisms. Patients do not suffer from fever, malaise or regional lymphadenopathy—suggesting that a product of the infection has local immunosuppressive and/or antiphagocytic activities. This hypothesis was tested by observing the effects *in vitro* of the mycobacterial toxin on T-cell proliferation and on phagocytosis assays. Both functions were impaired by a crude filtrate of toxin, the antimacrophage effect at higher concentrations of toxin. The mode(s) of suppression is speculative: T-cell interleukins or receptors may be the site of action; or macrophage functions in activating T cells. The possibility is not excluded that toxin in Buruli ulcers is in such high concentration (higher than tested in this study) that lymphocytes and macrophages are themselves killed. The authors speculate that in Buruli ulcer, the healing phase begins when *M. ulcerans* is no longer producing toxin, thus permitting development of the host response to other compoents (e.g., cell wall), with resulting granuloma formation.—S. B. Lucas (Trop. Dis. Bull.)

Piquero-Martín, J., Pérez-Alfonzo, R., Abrusci, V., Briceno, L., Gross, A., Mosca, W., Tapia, F. and Convit, J. Clinical trial with clofazimine for treating erythema dyschromicum perstans; evaluation of cell-mediated immunity. Int. J. Dermatol. **28** (1989) 198–200.

Eight patients were studied to determine the possible use of clofazimine for treating erythema dyschromicum perstans (EDP). The T-helper/T-suppressor cytotoxic ratio (CD-4/CD-8) and the *in vitro* lymphoproliferative response on stimulation with phytohemaglutin (PHA) and concanavalin A (ConA) were determined in peripheral blood before and after treatment. Of the 8 patients studied, 7 had excellent to good responses; whereas only 1 had a marginal response. The immunologic evaluation before and after treatment showed a significant change in the CD-4/CD-8 ratio, an increase in the response to PHA, and no change in the response to ConA. The results obtained show that clofazimine is useful for treating this nosologic entity because of its cosmetic effect, and also because it induces changes in cell-mediated response, which could be very important therapeutically.—Authors' Abstract

Rastogi, N., Blom-Potar, M.-C. and David, H. L. Comparative intracellular growth of difficult-to-grow and other mycobacteria in a macrophage cell line. Acta Leprol. 7 Suppl. 1 (1989) 156–159.

We have recently developed a murine macrophage cell line (J-774) model which permits the growth of various mycobacteria. The purpose of the present investigation was to compare the intracellular growth of various difficult-to-grow mycobacteria (*Mycobacterium paratuberculosis, M. ulcerans*), and other pathogenic (*M. tuberculosis* H37Rv, *M. kansasii, M. bovis*) and nonpathogenic or avirulent (*M. tuberculosis* H37Ra, *M. bovis* BCG, *M. gastri*) mycobacteria. Electron-microscopic studies were also performed to elucidate whether the formation of an electron-transparent zone around phagocytized bacilli was linked to their intramacrophagic survival. Furthermore, the comparison of intracellular growth of a pathogenic (*M. kansasii*) and nonpathogenic (*M. gastri*) mycobacteria, sharing the same phenolic glycolipid antigen at their surface (mycoside-A), suggested that these antigens did not play a primary role in intracellular survival and multiplication of these bacteria. Also, we were unable to propagate *M. ulcerans* inside J-774 macrophages, which were massively lyzed after infection (due to a characteristic toxin secreted inside the macrophages?). These results are discussed in terms of the validity of the J-774 model for studying intracellular growth of mycobacteria.—Authors' Summary

Rastogi, N. and Frehel, C. Resistance of *Mycobacterium avium* to microbicidal activities in bone-marrow macrophages from naturally susceptible (C57BL/6) and naturally resistant (DBA-2) mice. Acta Leprol. 7 Suppl. 1 (1989) 177–178.

In this study, the phagosome-lysosome fusion (PLF) inhibition and the presence of the electron-transparent zone (ETZ) around phagocytized *Mycobacterium avium* both in the naturally-susceptible and naturally-resistant mice were compared. We observed marked differences in case of these two mice strains, as PLF inhibition, as well as the presence of ETZ, was more important in case of naturally susceptible mice.—Authors' Summary

Salem, J. I., Gadelha, A. R., Maroja, F. and David, H. L. Non-cultivable mycobacteria in ulcers of the skin. Acta Leprol. 7 Suppl. 1 (1989) 10–15.

In biopsies of 54 patients suffering from chronic dermatological lesions (mostly ulcers of the skin) acid-fast bacilli were found in 14. Of these 14 cases in 4 were lesions caused by *Mycobacterium tuberculosis,* in 1 the lesion was caused by *M. avium-intracellulare,* in 1 the lesion was caused by *M. fortuitum* and in 2 the lesions were caused by noncultivable mycobacteria (Feldmann-Hershfield ulcers?). In 2 cases the cultures were heavily contaminated, and the diagnosis remained uncertain. In the remaining 4 cases the mycobacteria were considered occasional isolates without clinical significance.—Authors' Summary

Thurman, P. and Draper, P. Biosynthesis of phenolic glycolipids in *M. microti.* Acta Leprol. 7 Suppl. 1 (1989) 74–76.

Mycobacterium microti readily incorporates radioactive propionate into phenolic glycolipids and phthiocerol dimycocerosates. This process is inhibited by 2- and 3-fluoropropionic acids at concentrations which do not affect overall growth. Incorporation is also inhibited by N-(phosphonomethyl) glycine, an inhibitor of the synthesis of aromatic units, but only at high concentrations which also inhibit bacterial growth.—Authors' Summary

Tsukamura, M. and Miyachi, T. Correlations among naturally occurring resistances to antituberculosis drugs in *Mycobacterium avium* complex strains. Am. Rev. Respir. Dis. **139** (1989) 1033–1035.

In *Mycobacterium avium* complex strains, which were not exposed previously to any antituberculosis drugs, resistances to rifampin, minocycline, and kitasamycin, and resistances to streptomycin and kanamycin appeared frequently in the same strains. These data indicate that patterns of drug resistance in these organisms are not random but occur through linked mechanisms.—Authors' Summary

Williams, J. C., Nkoko, B., Pauwels, P., Karhahunga, C., Kaboto, M., Jeugmans, J., Burtonboy, G. and Prignot, J. [Tuberculosis and anti-HIV seropositivity in Kinshasa, Zaire.] Ann. Soc. Belg. Med. Trop. **68** (1988) 165–167. (in French)

This study examines the seroprevalence for HIV among sputum smear-positive new cases of tuberculosis presenting as outpatients to the main tuberculosis clinic in Kinshasa during a 3-month period in early 1987.

In contrast to the HIV prevalence rate of 33% among tuberculosis sanatorium patients in Kinshasa in 1985, 17% of 509 cases were found to be HIV positive in an ELISA screening test (Wellcozyme) confirmed by an in-house indirect immunofluorescence test in Brussels. HIV seroprevalence was significantly higher in women than in men: 47 (23.0%) of 204 compared with 38 (12.5%) of 305, $p = 0.002$.

The culture rate was generally high and similar in both HIV-positive and -negative groups. All isolations were of *Mycobacterium tuberculosis.*

The seroprevalence rate was said to be double that of a nontuberculous population in the same area in 1986, and [even more depressing] a preliminary follow-up study showed a 32.5% mortality among the HIV-seropositive patients compared with 1.5% among seronegative patients.—P. Nunn (Trop. Dis. Bull.)