CURRENT LITERATURE

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

General and Historical

Abe, H. R. [To the history of leprosy in Erfurt.] Dermatol. Mon. Schr. **176** (1990) 765–770. (in German)

In Erfurt, which was one of the largest cities in Germany in the Middle Ages, leprosy appeared from the beginning of the 13th century to the beginning of the 17th century. This appearance was typical for the development of this illness in Germany. The leprosy patients were isolated in special hospitals. The number of these hospitals varied between two and four.—From Author's English Summary

Friedericks, R. C. A multi-media approach to health education. Lepr. Rev. **62** (1991) 329–332.

The author discusses general principles of health education of the public. The Leprosy Control Program in Nepal has made significant progress in early case finding and public attitudes by the continuous use of the simple messages that leprosy can be cured, that the early signs are . . ., and that treatment can be found at . . . . These messages have been repeated by healthworkers in the field, distributed as printed material to community leaders, and broadcast by radio.—C. A. Brown (Trop. Dis. Bull.)

Isaacs, H. D. Text and documents; a medieval Arab medical certificate. Med. Hist. **35** (1991) 250–257.

Among the Geniza writings preserved in Cambridge University Library's Taylor-Schechter Collection is a medieval Arabic medical certificate which has not hitherto been published or studied. Class-marked as T-S NS 327.51, this document is the earliest medical certificate known to me and is of sufficient interest to merit publication. Since the condition to which the certificate relates is leprosy, I propose not only to publish the Arabic along with an English translation, but also to consider some of the relevant aspects of the medical history of leprosy.

In the name of God the Compassionate, the merciful.

Those who set their hand hereto and have fully declared their names, among those men in positions of trust whose word in their attestations is accepted hereby attest that they attended Ibrāhīm al-Yahūdī [i.e., Abraham the Jew], who has been affected by such black bile as has caused him to develop leprosy, and that fact is such that it debars him from mixing freely with the Muslims and from earning his living. Having ascertained the truth of the matter by their having attended and established an accurate diagnosis of his illness, and having been requested to issue an attestation of their finding, they have complied with the request, such attestation being issued on the first day of Rabī al-Ākhir of the year six hundred and sixty [23 February AD 1262].

Testimony

I attended the above named and found him to be suffering from leprosy. He may not mix freely with the Muslims because that condition is a transmissible and communicable disease.

Signed by Abū al-Tāhir b. al-Husayn.—From the article

Kloehn, G. Leprosy treatment in Africa with the Flying Doctor Service of East Africa. Rev. Med. Univ. Navarra **33** (1989) 81–87.

The Flying Doctor Service of East Africa and the African Medical and Research Foundation (AMREF) have been especially helpful in disseminating education along with associated primary and secondary

health care to leprosy-affected regions of the Third World for over 30 years. Reconstructive surgery has helped leprosy victims both functionally and psychologically, re-establishing their courage and belief in the fact that this dreaded social stigma need not affect their ability to hold a job, maintain personal relationships and lead an otherwise normal life within the community.—Author's Summary

Meyers, W. M. and Marty, A. M. Current concepts in the pathogenesis of leprosy; clinical, pathological, immunological and chemotherapeutic aspects. Drugs **41** (1991) 832–856.

In recent years there have been notable advances in the laboratory investigation and field management of leprosy. Progress, however, continues to be hindered by the lack of efficient methods for early diagnosis and implementation of control and treatment measures. Diagnosis is still made on the same principles as a century ago (clinical and histopathological findings), and only 1 in 3 known patients worldwide receives optimal chemotherapy. In 1988, nearly 1 in 10 newly diagnosed patients already had debilitating deformities. Contributing factors are operational, administrative, and financial difficulties in implementing multidrug therapeutic regimens, inadequately trained personnel, and lack of priority and political commitment to leprosy control.

The formulation and implementation of multidrug therapy is the most important development in leprosy in the past 10 years. Dapsone monotherapy was the mainstay for treatment and control for approximately 40 years, but secondary dapsone-resistant strains, first noted in 1964, now infect as many as 50% of all new patients. Multidrug regimens recommended by the WHO consist of various combinations of therapy using dapsone, rifampin, clofazimine and a thionamide. Duration of therapy is limited to 6 months for paucibacillary and 2 years or more for multibacillary patients; relapse rates thus far are low. The average cost of treatment worldwide, including the cost of drugs, is estimated at US$150 per patient. The recent annual drop of nearly 8% in newly registered patients may be due to the implementation of these therapeutic regimens. Newer drugs that may be introduced into these regimens include fluoroquinolones, minocycline and clarithromycin.

While knowledge of the microbiology of the leprosy bacillus and host response has advanced remarkably, there is little improvement in the understanding or amelioration of social aspects of leprosy. Better treatment and control reduces the stigma, but improvements in the attitudes of patients and society towards leprosy are as important as advances in medical science in achieving ultimate eradication of the disease.—Authors' Summary

Noordeen, S. K. Leprosy control through multidrug therapy (MDT). Bull. WHO **69** (1991) 263–269.

This article in the Update series is presented by the Chief Medical Officer of WHO's Leprosy Unit.

Coverage of leprosy patients with multidrug therapy (MDT) had reached 55.7% of the total number of registered cases by October 1990. However, the distribution of MDT among the WHO regions has been very uneven, being particularly low in Africa (18% coverage of registered cases) and the Americas (24%) compared with S.E. Asia (66%) and the Western Pacific (63%).

The author predicts that the increasing political commitment in many countries in terms of an appreciation of the value of MDT, together with increasing international cooperation, could reduce the number of leprosy cases globally by as much as 90% over the next 10 years. He cautions that future leprosy control programs within primary health care will still be needed to provide early detection of new disease resulting from infection several years earlier, continued treatment of existing patients, and disability prevention and management among cured patients.—C. A. Brown (Trop. Dis. Bull.)

Ramirez-Soltero, S., Aguirre-Negrete, M. G. and Padilla-Gutierrez, L. [The level of knowledge about leprosy among university students.] Salud Publica Mex. **32** (1990) 583–588. (in Spanish)

A survey about leprosy was made in 1000 students from the University of Guadala-

jara (Guadalajara, Jalisco, Mexico). There were considered clinical, preventive, social and etiological aspects. The results in this group considered to represent the new generation showed that the patient suffering from leprosy is currently stigmatized. We suggest that this study should be carried out in other universities in Mexico with the purpose of verifying the stigmata of this entity.—Authors' English Abstract

Thomsen, R. J. How leprosy is depicted in *Ben-Hur.* Int. J. Dermatol. **30** (1991) 818–824.

There are two areas in which we may take issue with Governor Wallace. The first is with his description of modern leprosy, for this is clearly what he envisioned. Several obvious inaccuracies enhance the horror of the disease and have perpetuated deeply ingrained social reactions against those with leprosy. We also take issue with the assumption that the diseases referred to as *tsara'at* and mistranslated as "leprosy" in the Bible were identical to what we now call leprosy. It is clear that this is an incorrect assumption, although it is still commonly believed. Governor Wallace can be forgiven for portraying leprosy with such dramatic force in the manner in which it was then generally perceived. Nevertheless, the popularity of *Ben-Hur* in the book, play, and movies has perpetuated the stigma associated with leprosy and preserved the widespread confusion of Biblical *tsara'at* with modern leprosy.—Author's Conclusions

Wood, S. R. A contribution to the history of tuberculosis and leprosy in 19th century Norway. J. R. Soc. Med. **84** (1991) 428–430.

The contrasting incidence and distribution of tuberculosis and leprosy in 19th century Norway suggests either a fortuitous inverse relationship between them or, more probably, one based on cross immunity; in either case independent of commonly accepted socioeconomic factors.—From the article

Chemotherapy

Carrazana Hernández, G. B. and Castaño Hernández, S. T. [Adverse, nonsevere side effects seen in leprosy patients implementing multidrug therapy.] Rev. Leprol. Fontilles **18** (1991) 145–151. (in Spanish)

A study on nonsevere, adverse side effects seen during the first 9 months of implementation of multidrug therapy with rifampin, dapsone and clofazimine was made on 57 patients (96.6%) of leprosy prevalence at the health center "Centro" in Camagüey city, Cuba, during 1989. The percentage of cases with nonsevere, adverse side effects was high (43.9%). Weakness, anorexia, cutaneous and mucous paleness, and ichthyosiform skin were the signs and symptoms more frequently observed. The laboratory findings corresponding to the anemia were the more relevant ones.—Authors' English Summary

Chen, L.-F., *et al.* [Effects of MDT on leprosy in Wuhan area.] China Lepr. J. **7** (1991) 72–76. (in Chinese)

In Wuhan City with 10 counties and one city, 190 active cases of leprosy have been taking MDT, of which 163 cases have completed the course of the treatment, including 76 cured (46.6%) and 78 ceased treatment in line with Chinese criteria for ceasing treatment (47.9%). Only nine MB cases do not conform with the criteria after the 2-year treatment (5.5%), but still show some improvement clinically and bacteriologically. Of 120 MB cases of leprosy completing 2 years of treatment, 74 cases presented negative skin smears (61.7%). The authors think that WHO MDT can prevent drug resistance and shorten the course of treatment, and the criteria for stopping drugs according to MDT regulation set by the

Ministry of Public Health is reasonable.—Authors' English Abstract

Dhople, A. M., Lamoureux, L. C. and Gardner, G. D. *In vitro* and *in vivo* interactions of drugs used in multidrug therapy in leprosy. Indian J. Lepr. **63** (1991) 166–179.

Interactions of different drugs commonly used in multiple drug therapy were evaluated using both *in vitro* culture (cell-free as well as macrophage) system and mouse foot pad. No additive effects were obtained in the *in vitro* system when dapsone was combined with either rifampin or clofazimine, while a strong antagonism was observed when clofazimine was combined with rifampin but not with rifabutin. In the mouse foot pad system, a strong synergism was obtained when clofazimine was combined with either rifampin or rifabutin, but significant antagonism was observed with the combination of clofazimine and dapsone.—Authors' Abstract

Garrelts, J. C. Clofazimine: a review of its use in leprosy and *Mycobacterium avium* complex infection. DICP Ann. Pharmacother. **25** (1991) 525–531.

This article reviews the chemistry, pharmacology, spectrum of activity, pharmacokinetics, clinical efficacy in leprosy and *Mycobacterium avium* complex (MAC) infection, adverse effects, drug interactions, and special considerations of clofazimine. The drug is active *in vivo* against *M. leprae* and *in vitro* against MAC. In addition, it possesses antiinflammatory and immunosuppressive properties. Clinical studies support the efficacy of clofazimine as a part of multidrug therapy in treating leprosy. It also appears to reduce the incidence and severity of erythema nodosum leprosum reactions that often occur during the treatment of leprosy. Efficacy in treating MAC infection in patients with AIDS is not well documented, despite the use of clofazimine in combination with other agents. A few patients have responded symptomatically and by clearing their mycobacteremia, although there is no evidence that mortality is reduced. Clofazimine is well tolerated, at least when doses $\leq$ 100 mg/d are used. Adverse reactions include discoloration of the skin, self-limiting gastrointestinal intolerance, severe and life-threatening abdominal pain and organ damage due to clofazimine crystal deposition, and asymptomatic discoloration of the eye. Clofazimine should be considered for formulary inclusion.—Author's Abstract

Gelber, R. H. The activity of amoxicillin plus clavulanic acid against *Mycobacterium leprae* in mice. J. Infect. Dis. **163** (1991) 1374–1377.

The activity of amoxicillin plus clavulanic acid against logarithmically multiplying *Mycobacterium leprae* was evaluated by treating mice by gavage five times weekly with various amounts of the compound from day 60 to day 150 after foot pad infection. At 25, 50, and 100 mg/kg, it was inactive; at 200–600 mg/kg, multiplication of *M. leprae* was entirely prevented for 6–11 months after drug discontinuation, consistent with observations of bactericidal activity for *M. leprae*. In a confirmatory study in mice, five-times-weekly intraperitoneal ticarcillin plus clavulanic acid, 1000 mg/kg, was not bactericidal for *M. leprae*, while amoxicillin plus clavulanic acid, 400 mg/kg five times weekly, was weakly bactericidal (80% $\pm$ 14%). In addition, activity of amoxicillin plus clavulanic acid, 400 mg/kg, was evaluated in combination with previously established active drugs dapsone, 0.0001% (in diet), rifampin, 20 mg/kg monthly (by gavage), and kanamycin, 25 mg/kg five times weekly (intraperitoneally). All three combinations were active, and the combination with kanamycin was more active than either drug alone.—Author's Abstract

Hu, L.-F., *et al.* [Results of monitoring 657 ex-patients with MB leprosy in the first year after stop of MDT.] China Lepr. J. 7 (1991) 133–136. (in Chinese)

Six-hundred-fifty-seven cases of MB leprosy treated with WHO's (1981) MDT regimen over 2 years and predetermined to be monitored for 5 years after stop of the treatment have already been monitored over 1 year. By the end of the first year of monitoring, in 42 originally new cases with active skin lesions before MDT, most of the skin lesions resolved, and the bacterial index (BI) decreased from 3.07 to 0.61 with a yearly

decline of 0.82 and 10 skin-smear negative cases (23.8%). In 615 cases treated with antileprosy drugs and who had active skin lesions in about half of them before MDT, all of the skin lesions resolved and the BI decreased from 1.55 to 0.2 on the average with yearly decline of 0.45 and 436 skin smear negative cases (70.89%). In 3 years of MDT and monitoring, ENL in 96 cases and neuritis in 125 cases were found and their number and severity degree have been decreasing as time goes on. Type 1 reaction in 32 cases was mostly seen in the period of the 2nd to 12th months of taking MDT and disability derived from leprosy reaction might be prevented with prednisone given in time. Pigmentation and ichthyosis-like lesions of the skin caused by clofazimine would gradually resolve as time goes on after stop of MDT. The authors think that the WHO's MDT regimen is adoptable in peripheral mountain regions because the patients like to take it. Relapse rate in these cases has yet to be determined.—Authors' English Abstract

Ji, B. and Grosset, J. Ofloxacin for the treatment of leprosy. Acta Leprol (Genève) **7** (1991) 321–326.

Among the major commercially available fluoroquinolones, ciprofloxacin was inactive against *Mycobacterium leprae* in mice; pefloxacin was active, 50 mg/kg daily showed bacteriostatic activity but 150 mg/kg daily displayed bactericidal activity; ofloxacin was more active than pefloxacin, 50 mg/kg daily exerted the same level of bactericidal effect as pefloxacin 150 mg/kg daily, and ofloxacin 150 mg/kg displayed profound killing activity. Two clinical trials with 6 months of pefloxacin and/or ofloxacin in 31 previously untreated lepromatous patients have been completed. Pefloxacin 400 mg twice daily or 800 mg once daily or ofloxacin 400 mg once daily were equally effective; definite clinical improvement with drastic decrease of morphological index to the baseline were observed in all patients at 2 months after beginning of treatment; about 99.99%, or 4 "logs," of organisms viable on day 0 were killed by 22 doses of either pefloxacin or ofloxacin. The side effects from the two trials were rare and mild, and the patients tolerated extremely well the combinations of pefloxacin/ofloxacin plus multidrug therapy (MDT) regimen for multibacillary leprosy recommended by WHO. The amount of rifampin-resistant mutants in lepromatous patients before treatment is no more than 4 "logs," thus, all rifampin-resistant mutants may be eliminated by 22 doses of either pefloxacin or ofloxacin. It is, therefore, possible that the combination of ofloxacin and rifampin may considerably shorten the required duration of MDT.—Authors' Summary

Oommen, T., Natu, M. V. and Wilfred, D. Comment: clofazimine-induced lymphoedema. (Letter) Lepr. Rev. **62** (1991) 341–342.

As a follow-up to a previous letter about clofazimine-induced lymphedema (Leprosy Review, 1990, 61, 289) these correspondents report their observations for 45 patients with pedal edema seen among 75 leprosy patients selected at random at a regular outpatient clinic in India. The edema was varied in nature and possibly of different etiologies. However, the correspondents propose that in 10 of the 45 patients the pedal edema was due to a lymphatic stasis produced by clofazimine in the lymphatic channels. They call for information regarding similar observations after clofazimine treatment from readers.—C. A. Brown (Trop. Dis. Bull.)

Pandian, T. D., Muliyil, J. and Vellut, C. Risk of relapse among non-lepromatous patients released from treatment after dapsone monotherapy. Lepr. Rev. **62** (1991) 288–296.

Information on 14,625 nonlepromatous patients released from treatment after dapsone monotherapy and followed up to a maximum of 15 years at the ILEP project, Dharmapuri, India, was analyzed to study the pattern of relapses. The overall relapse rate was 5/1000 person years. Males had a higher relapse rate than females. The risk of relapse increased with age, number of lesions and duration of treatment. The risk for relapse remained constant over several years after release from treatment. Even though the absolute risk for relapse after MDT may be different, the pattern of re-

lapses and the factors affecting it may be similar to what has been shown in this study.—Authors' Summary

Sahu, A., Saha, K., Banerjee, N. R., Sehgal, V. N. and Jagga, C. R. Effect of antileprosy drugs on superoxide anion production by rat peritoneal macrophage with special reference to light exposed clofazimine. Int. J. Immunopharmacol. **13** (1991) 419–428.

The present study describes the *in vitro* effect of antileprosy drugs on superoxide anion (O_2^-) production by rat resident peritoneal macrophages. Of the three drugs tested, i.e., clofazimine, rifampin and dapsone, the first was most effective in increasing O_2^- production in a dose-dependent manner, while rifampin had some stimulatory effect and dapsone exhibited minimal action. Furthermore, when clofazimine and dapsone were added together it was observed that the increase of O_2^- production by macrophages due to clofazimine was not significantly altered by the addition of dapsone. Moreover, it was found that killed *Mycobacterium leprae* could induce a lesser amount of O_2^- production in comparison to that of *Staphylococcus aureus* and the enhancement of O_2^- release due to clofazimine was stimulus dependent. This increase of O_2^- release after addition of clofazimine was inhibited by the addition of *p*-bromophenacyl bromide. Another interesting finding was that the enhancement of O_2^- production by clofazimine gradually decreased as clofazimine was exposed to light for days. On further investigation it was found that ultraviolet, NMR, infrared and mass spectra of the light unexposed and exposed drug were similar, but the diffusion current of the polarogram of light exposed drug was remarkably more than that observed in light unexposed drug, indicating thereby a possible increase in the electron accepting capacity of the light reacted molecule. As far as we know, this is the first report describing the effect of light-exposed clofazimine on the respiratory burst activity of macrophages.—Authors' Abstract

Walker, R. C. and Wright, A. J. The fluoroquinolones. Mayo Clin. Proc. **66** (1991) 1249–1259.

The fluoroquinolone class of antibiotics promises to become as diverse and as important as β-lactam agents. The fluoroquinolones inhibit bacterial DNA gyrase and are bactericidal. All fluoroquinolones have potent activity against most gram-negative bacteria; ciprofloxacin is the most active against *Pseudomonas aeruginosa.* Activity against gram-positive organisms is variable; methicillin-resistant *Staphylococcus aureus* has acquired resistance to the fluoroquinolones at an alarming rate. Currently available quinolones do not have, but new quinolone agents likely will have, substantial activity against anaerobic bacteria. Some quinolones are also active against *Mycobacterium, Chlamydia,* and *Mycoplasma* organisms. All fluoroquinolones have excellent absorption after oral administration; however, this process can be impaired by the presence of aluminum- or magnesium-containing antacids and by zinc, iron, or calcium supplements. Ciprofloxacin is also available for intravenous use. Although most fluoroquinolones do not achieve adequate cerebrospinal fluid levels, penetration into other tissues is excellent. Dosage adjustments for renal and hepatic dysfunction vary among the quinolones. Although side effects are rare, concomitant use of caffeine or theophylline with some quinolones may cause toxicity to the central nervous system. Because they may affect the development of cartilage, all fluoroquinolones are contraindicated in children, adolescents, and pregnant or breast-feeding women.—Authors' Abstract

Wang, H., Li, W., Ye, G., Yu, L. and Shi, M. The susceptibility testing of 13 strains of *Mycobacterium leprae* to rifampicin and the determination of minimal effective dosage. Lepr. Rev. **62** (1991) 276–279.

By use of the mouse foot pad technique, the susceptibility testing of 13 strains of *Mycobacterium leprae* to rifampin (RFP) and the determination of minimal effective dosage (MED) were carried out. Among these strains of *M. leprae,* 8 were obtained from previously untreated multibacillary leprosy patients and 5 from relapsed leprosy patients without using RFP previously. The results showed that the MED of all strains

to RFP were ≤ 0.001% RFP in the diet, 5 strains being equal to 0.001%, 5 ≤ 0.0001%, 2 ≥ 0.0003% and 1 ≤ 0.0003%. The results indicated that the MED value of RFP could be lower than that of other reports. Because the critical concentration of RFP for assessment of RFP-resistant strains is not well established a further study would be worthwhile. The results of the determination of sera RFP concentrations in mice administered the RFP diet were identical with that of Holmes' report. Five of the 13 strains also showed that the growth of bacilli were suppressed by 10 mg/kg RFP using the gavage method.—Authors' Summary

Yoder, L. J., Guitrau, M. and Jacobson, R. Comparison of a urine spot test and blood tests as indicators of patient compliance. Indian J. Lepr. **63** (1991) 195–202.

Irregular drug intake has been a concern of leprosy control programs for many years, and various methods have been used to monitor and encourage patient compliance. This study compares the results of a urine spot test for dapsone as proposed by Huikeshoven, with blood levels measured in the same patients by the modified Bratton Marshall method and by high-performance liquid chromatography. Two-hundred-sixty urine and blood specimens were obtained from subjects who were taking supervised and unsupervised medications as well as from controls who were taking no medications. The results indicate that the urine spot test is simple and easily performed, and for monitoring patient compliance under routine clinical conditions (hospital or field work) it compares favorably with blood levels of dapsone estimated by the Bratton Marshall method or by high-performance liquid chromatography. The study also shows that dapsone level is not a good indicator of compliance in patients who are also taking daily rifampin but the urine spot test remains useful in such patients.—Authors' Abstract

Yoder, L. J., Jacobson, R. R. and Hastings, R. C. The activity of rifabutin against *Mycobacterium leprae.* Lepr. Rev. **62** (1991) 280–287.

Minimal effective doses of rifabutin and rifampin were determined in *Mycobacterium leprae* isolated from skin biopsies of newly diagnosed, previously untreated lepromatous leprosy patients. Rifabutin was more potent than rifampin. Our previous report that rifabutin was fully active against rifampin-resistant *M. leprae* could not be confirmed. Examination of two strains of rifampin-resistant *M. leprae* from elsewhere, and a repeat experiment on our original strain of rifampin-resistant bacilli, showed full crossresistance between rifampin and rifabutin. A clinical trial in three newly diagnosed, previously untreated lepromatous patients showed that rifabutin has rapid bactericidal activity.—Authors' Summary

Zhou, D.-H., *et al.* [Observation on retreatment with MDT in 657 cases of MB leprosy cured with DDS.] China Lepr. J. **7** (1991) 136–138. (in Chinese)

To reduce the relapse of leprosy, 657 MB leprosy patients cured with dapsone alone have accepted MDT for a year, including rifampin and clofazimine each 1200 mg monthly supervised and dapsone 100 mg daily by patients, of which 620 cases (94.27%) have regularly finished the course and 548 (83.4%) were followed up for more than 5 years without relapse.—Authors' English Abstract

Clinical Sciences

Achenbach, R. E., Diez, A. and Di Fabio, N. [Lepromatous leprosy; an unusual form of presentation.] Rev. Argent. Dermatol. **72** (1991) 135–138. (in Spanish)

A 34-year-old man with an unusual form of lepromatous leprosy is presented. He complained only of a few papular lesions (microlepromata) localized to an area of his

right thigh. The rest of the skin was normal. The lesions had a high bacterial index while most of the body surface was negative or almost negative bacteriologically. Smears of the nose were also negative. Histopathology showed a lepromatous granuloma with solid bacilli and a subepidermal clear zone. The additional clinical diagnosis was sarcoidosis or tuberculoid leprosy. There was a mild loss of sensation to light touch and temperature. On the other hand these lesions were not anesthetic and there was no enlargement of peripheral nerves. Multidrug therapy resulted in resolution of the lesions with residual hyperpigmented macules.—Authors' English Summary

Al-Majed, S. A. Lepromatous leprosy in a Saudi female from central region. Emirates Med. J. **8** (1990) 227–229.

The author gives an excellent account of lepromatous leprosy in a 70-year-old woman in Saudi Arabia who was admitted to hospital for obstructive airways disease. She had been treated with dexamethasone and required further treatment, in the intensive-care unit, with hydrocortisone 100 mg 6-hourly, later rising to 200 mg 6-hourly. Three days after her condition stabilized, she developed a fever accompanied by bilateral symmetrical skin nodules on the upper and lower extremities, clinically typical for erythema nodosum leprosum (ENL) complicating lepromatous leprosy. Skin smears were positive and histopathological examination of skin biopsies, including acid-fast stains, was confirmative. She responded well to triple drug therapy with dapsone, clofazimine and rifampin and her reaction was controlled with prednisolone. The author draws attention to the likelihood that the clinical presentation of lepromatous leprosy in reaction in this case was triggered by the use of steroids, backed by the immunosuppression which commonly occurs at this age, and to the importance of watching out for presentations of this kind particularly in areas of low endemicity.—A. C. McDougall (Trop. Dis. Bull.)

Baconcini, C. [Psychopathological aspects in Hansen's disease.] G. Ital. Dermatol. Venereol. **125** (1990) 353–356. (in Italian)

Hansen's disease is an invalidating disease. The stigma of leprosy causes psychological problems both in the patient and the community. The most frequent psychopathological aspects in leprosy patients are discussed here.—Author's English Abstract

Butany, J. W., McAuley, P., Bergeron, C. and MacLaughlin, P. Giant cell myocarditis and myositis associated with thymoma and leprosy. Can. J. Cardiol. **7** (1991) 141–145.

Myocarditis is an inflammatory form of heart disease which is usually preceded by a viral infection. Giant cell myocarditis is an uncommon and nonspecific form of this disease. Sporadic reports have linked giant cell myocarditis with thymoma and concomitant myositis. The authors report a patient with leprosy who, 6 months after initiation of treatment, developed sudden onset of congestive heart failure and cardiac arrhythmias unresponsive to aggressive medical therapy. In addition to confirming leprosy, autopsy showed a mixed cell type thymoma, severe giant cell myocarditis and extensive myositis.—Authors' Abstract

DeFaria, C. R. and Silva, I. M. Electromyographic diagnosis of leprosy. Arq. Neuro-Psiquint. (São Paulo) **48** (1990) 403–411.

Eighty untreated patients suspected to have leprosy were submitted to neurophysiological examination and later compared with the clinical diagnosis. Among the patients who had leprosy confirmed, 98% had EMG abnormalities. Motor and sensory amplitude reduction was the earliest and the most frequent abnormality. Low conduction velocity of the ulnar nerve across the elbow was present in over 55% of the patients. A "mosaic" peripheral polyneuropathy was the most characteristic finding, and seems to be helpful to the diagnosis of leprosy. All of the clinical forms showed EMG abnormalities, and even some asymptomatic contacts, however the abnormalities increased from the indeterminate and tuberculoid to the borderline and Virchow's forms.—Authors' Summary

Delabie, J., De Wolf-Peeters, C., Bobbaers, H., Bilbe, G. and Desmet, V. J. Immu-

nophenotypic analysis of histiocytes involved in AIDS-associated *Mycobacterium scrofulaceum* infection: similarities with lepromatous lepra. Clin. Exp. Immunol. **85** (1991) 214–218.

The present study reports a rare case of systemic *Mycobacterium scrofulaceum* infection in an AIDS patient and analyzes the inflammatory infiltrate in a lymph node by immunohistochemistry. Special emphasis is put on the histiocytes. The diffuse infiltrate consists mainly of large histiocytes that contain numerous bacili. These cells display the phenotype of mature histiocytes and, in addition, coexpress the antigens recognized by RFD7 and RFD9, both markers of different subsets of histiocytes which have been reported to be co-expressed by the infected histiocytes in the infiltrate of lepromatous leprosy. Interdigitating reticulum cells are rare as well as T cells which are mainly of the suppressor/cytotoxic type. These findings are similar to those reported for lepromatous leprosy and might indicate common deficiencies in T cell-macrophage interactions in both conditions. Superimposed on the diffuse infiltrate of large histiocytes we observed "monocytic granulomas," the presence of which might be related to a reactional state comparable to erythema nodosum leprosum, a reactional state of lepromatous leprosy.—Authors' Summary

Graninger, W., Hollenstein, U., Dudeck, U., Kremsner, P. G. and Savlan, T. [Autoantibodies in lepromatous leprosy.] Wien. Klin. Wochenschr. **103** (1991) 268–270. (in German)

Eighty-four Turkish patients with lepromatous and borderline lepromatous leprosy were investigated with regard to the presence of antibodies to a variety of tissue constituents and immunoglobulins. Thirty Turkish-born volunteers and two groups of patients with systemic lupus erythematosus and primary biliary cirrhosis, respectively, served as controls. No increased incidence of autoantibodies to smooth muscle cells, nuclei, mitochondria, striated muscle, thyroid microsomes and thyroglobulin was detected. A high incidence of rheumatoid factor and circulating immune complexes was found.—Authors' English Summary

Husain, S., Malaviya, G. N., Girdhar, A., Sreevatsa and Girdhar, B. K. Nasal myiasis in leprosy. Lepr. Rev. **62** (1991) 389–394.

Infestation of the nose with larvae of certain flies can occur in leprosy patients. This results in severe distress and agony and can cause extensive tissue damage. The predisposing factors, clinical presentation, and treatment are described.—Authors' Summary

Malin, A. S., Waters, M. F. R., Shehade, S. A. and Roberts, M. M. Leprosy in reaction: a medical emergency. BMJ **302** (1991) 1324–1326.

Leprosy is a chronic granulomatous infectious disease that primarily affects the skin and nerves. The progress of the disease is often considered to be slow and indolent. However, skin or nerve lesions may suddenly flare up in a state of immunological reaction (lepra reaction), of which two main types are recognized: reversal (upgrading) reaction (type 1 reaction) and erythema nodosum leprosum (type 2 reaction). Delayed treatment of these reactions may result in irreversible nerve damage. The three cases described illustrate the difficulties in diagnosis and the problems that may ensue if lepra reactions are not promptly recognized and treated.—From the article

Nigam, P. K., Gupta, S. R. and Nair, A. Leprosy and psoriasis occurring together. (Letter) Int. J. Dermatol. **30** (1991) 676.

The association between leprosy and psoriasis had not been reported previously and was attributed to highly activated reticuloendothelial and phagocytic systems in psoriatics. The report on leprosy and psoriasis occurring together by Sugathan and Riyaz postulated that there may be an HLA antigen common to psoriasis and leprosy associated with a defective T-cell gene. Only such patients will have both diseases. We are following one such case of leprosy in which psoriasis later developed.—From the letter

Okoro, A. N. Pre-emptive diagnosis of leprosy. Int. J. Dermatol. **30** (1991) 767–771.

New cases of leprosy will continue to be found principally in hyperendemic and endemic regions (areas with high prevalence rates) among household and other contacts of multibacillary (infectious) patients, children in these regions, and subjects in the Hansen anergic fringe. The zone of new cases is fairly distinct. We must direct our preemptive diagnostic "strike" to this zone to pick up new or potential cases of leprosy during the latency period and offer early treatment to new cases and immunoprophylaxis to potential cases. By doing this we can put a stop to the neural and other complications that follow late diagnosis and late treatment and that perpetuate the stigma, dread, and horror associated with leprosy. Our mission is as follows: to examine with a high index of suspicion the high-risk individuals in these high prevalence rate regions, to pay special attention to children who are particularly susceptible, to track *Mycobacterium leprae,* and to track the tissue immunologic reactions and the body's physiologic responses using the expanding battery of *in vitro* and *in vivo* tests that are adding to the understanding of the variable and previously enigmatic latency period of leprosy.—Author's Conclusions

Patil, S. A., Tyagi, P., Katoch, K., Sreevatsa and Sengupta, U. Antigens of *Mycobacterium leprae* in the cerebrospinal fluid of leprosy patients: detection by monoclonal-antibody-based sandwich immunoradiometric assay and avidin/biotin immunoblotting. Clin. Exp. Immunol. **84** (1991) 515–521.

Mycobacterium leprae antigens could be detected in the cerebrospinal fluid (CSF) of patients with leprosy, using a monoclonal-antibody-based sandwich immunoradiometric assay (SIRMA). Antigens of 12 kDa, 35 kDa and 30–40 kDa were detected using ML06, ML04, and ML34 monoclonal antibodies, respectively. The 30–40-kDa polysaccharide antigen, although present in larger amounts in *M. leprae* than the 12-kDa and 35-kDa protein antigens, was found in the CSF of comparatively fewer subjects. The antigen capture assay has been found sensitive to the level of nanograms. Avidin-biotin-based immunoblotting using pooled leprosy sera detected a larger number of antigens than using anti-*M. leprae* antisera raised in rabbits. The immunoblotting of CSF samples revealed about three antigens in the region of 100–160 kDa and the three more in the region of 45–60 kDa as probed by leprosy sera. This study has for the first time revealed the presence of *M. leprae* antigens in the CSF of leprosy patients and the probable involvement of the central nervous system in leprosy.—Authors' Summary

Patki, A. H. and Baran, R. Significance of nail changes in leprosy: a clinical review of 357 cases. Semin. Dermatol. **10** (1991) 77–81.

Leprosy can cause many nail changes, which have been observed in up to 64% of infected patients. The manifestations of leprosy (clinical, bacteriologic, and histological) are profoundly affected by the patient's immunological status, which also determines the prognosis. Nail changes in leprosy can be caused by neuropathy and trauma, vascular impairment, infections, and miscellaneous changes. Often more than one factor will be important. Paradoxically, nail changes in tuberculoid and lepromatous patients are similar, despite wide differences in pathology. This may be because etiological factors common to both are implicated. Factors only associated with lepromatous disease are invasion of the bones of terminal phalanges by lepromatous granulomas and endarteritis occurring during type 2 lepra reactions. Otherwise, the only difference from tuberculoid leprosy is the time of onset and the symmetry of lesions. Lepromatous patients develop nail changes late in the course of disease. The presentation is usually bilaterally symmetrical. However, these changes are not specific to leprosy, and may be observed in other peripheral neuropathies.—Authors' Abstract

Rao, P. S., Ekambaram, V., Reddy, B. N., Krishnamoorthy, P., Suresh Kumar, S. K. and Dutta, A. Is bacteriological examination by skin smear necessary in all paucibacillary leprosy patients in mass control programmes? Lepr. Rev. **62** (1991) 303–309.

Skin smear bacteriological examination results of 11,255 paucibacillary leprosy pa-

tients from eight leprosy control units under the National Leprosy Eradication Programme (NLEP) in South India and the Outpatient Department (OPD) of the Central Leprosy Teaching & Research Institute (CLT&RI), Chengalpattu, between 1987 and 1989 were collected and analyzed. Only 0.05% of the smears from leprosy control units and 2.49% from the OPD of CLT&RI were found to be positive. Not a single smear from indeterminate, tuberculoid and pure neuritic types of leprosy out of 8263 examined was found positive under field conditions. The relevance of carrying out routine bacteriological examination in mass leprosy control programs is discussed.—Authors' Summary

Richardus, J. H. and Smith, T. C. Squamous cell carcinoma in chronic ulcers in leprosy: a review of 38 consecutive cases. Lepr. Rev. **62** (1991) 381–388.

The histories of 38 consecutive cases of squamous cell carcinoma (SCC) arising in chronic ulcers of leprosy patients treated between 1981 and 1990 at the McKean Rehabilitation Centre, Northern Thailand, were analyzed retrospectively. The study included 37 individual patients; 29 males and 8 females. The average age was 60 years, the average duration of leprosy was 34 years, and the average duration of ulcers was 12 years. Most patients (76%) came from leprosy settlements. Patients with borderline-tuberculoid (BT) leprosy were most commonly affected (63%), followed by lepromatous (LL) leprosy (21%), and borderline-lepromatous (BL) leprosy (16%). Four patients (11%) had histories of SCC on other extremities. Metastatic spread was observed in 2 cases (5%), both instances leading to death. The commonest site of involvement of SCC was the foot, but it was seen on the knee in 1 patient and on the hand in 2 others. The incidence rate of SCC in the group at risk (leprosy patients with disability grading 1 and 2) is calculated as being 0.79:1000 per year. SCC was seen in 1.8% of all cases admitted for ulcer care at the Centre. Treatment is by radical amputation. SCC in chronic ulcers in leprosy patients cannot be considered rare and emphasizes the need for an active policy of disability prevention in leprosy programs.—Authors' Summary

Roche, P. W., Theuvenet, W. J. and Britton, W. J. Risk factors for type-1 reactions in borderline leprosy patients. Lancet **2** (1991) 654–657.

Type-1 or reversal reactions are the major cause of nerve damage and disability in leprosy. [The authors] wished to determine whether there were any clinical or laboratory markers that identified patients with an increased risk of type-1 reaction. Forty-two (31%) of 136 Nepalese borderline leprosy patients (97 male, 39 female; age range 7–73 years) had a type-1 reaction during the first 2 years of multidrug therapy. Before therapy, 41 (98%) of the 42 patients were seropositive for antibodies to 1 of 3 mycobacterial antigens. Seropositivity for IgM anti-phenolic-glycolipid-1 (PGL-1) antibodies, but not IgG anti-lipoarabinomannan or anti-*Mycobacterium leprae* 35-kDa protein antibodies was significantly associated with subsequent manifestation of a type-1 reaction ($p < 0.001$). The concentration of IgM anti-PGL-1 antibodies in serum was significantly higher in patients in whom a type-1 reaction developed. The risk attributable to anti-PGL-1 seropositivity was independent of leprosy class, skin-smear positivity, and the presence of other anti-*M. leprae* antibodies (adjusted odds ratio = 8.7, $p < 0.001$). In the 87 patients who had a lepromin test, anti-PGL-1 seropositivity and lepromin reactivity were significant independent risk factors for subsequent reaction. Seventy-eight percent of patients with positive lepromin reactivity and IgM anti-PGL-1 antibodies had type-1 reactions. Patients with these risk factors should be carefully monitored during antimicrobial therapy to permit early initiation of anti-inflammatory treatment thus minimizing permanent nerve damage and resultant disability.—Authors' Summary

Salafia, A. and Menezes, M. Sulpiride and type II reaction. Rev. Leprol. Fontilles **18** (1991) 165–171.

The authors report on their experience with sulpiride, an antidepressant agent, in the control of type 2 reaction. Sulpiride has

been used in five patients with type 2 reaction. The severity of reaction was assessed by using a quantification method proposed by the first author in a separate paper. By this method it is easier to evaluate the efficacy of a drug as we demonstrate here.—Authors' Summary

Salafia, A. and Shah, J. K. ENL quantification—a proposal. Rev. Leprol. Fontilles **18** (1991) 173–177.

The authors propose a method of quantification of type 2 reactions, which will give to the clinicians and research workers common parameters for evaluating the severity of a reaction as well as the criteria for assessing the efficacy of a given drug.—Authors' Summary

Spindler, E., Deplus, S. and Flageul, B. [Acute uveitis in the course of reversal reactions.] Acta Leprol. (Genève) **7** (1991) 331–334. (in French)

Two cases of acute uveitis have been reported in reversal reactions in lepromatous patients treated with antihansenian multidrug therapy with daily rifampin. This type of eye damage has seldom been reported in reversal reactions.—Authors' English Summary

Tekle-Haimanot, R., Frommel, D., Tadesse, T., Verdier, M., Abebe, M. and Denis, F. A survey of HTLV-I and HIVs in Ethiopian leprosy patients. AIDS **5** (1991) 108–110.

We studied the HTLV-1 and HIV status in Ethiopian leprosy patients between October 1988 and September 1989. The infection rates for HTLV-1 were low among leprosy patients (0.4%), among nonleprosy dermatology patients (0.8%), and among healthy controls (0%). Antibodies to HIV-1 were found in 3.2% of leprosy patients, 2.8% of nonleprosy dermatology patients, and 2.5% of healthy controls.—From the article

Uplekar, M. W. and Cash, R. A. The private GP and leprosy: a study. Lepr. Rev. **62** (1991) 410–419.

In urban and rural areas alike, people in India tend to prefer private medical care to the existing government health services. Nevertheless, the large private health care sector has hitherto been virtually alienated from activities of public health importance, including priority disease control programs. This study of 106 private general practitioners (GPs), practicing in low socioeconomic areas of Bombay, shows a gross lack of knowledge and awareness among private doctors about leprosy and also about the National Leprosy Control Programme. The possible reasons are discussed. Effective involvement of GPs in the National Leprosy Control Programme should facilitate both integration and better implementation of leprosy control activities. The study also highlights some areas for future interventions at both primary and secondary health care levels and the need for a strategy, based on larger studies, to train and make private doctors participate in controlling diseases of major public health concern such as leprosy.—Authors' Summary

Walton, R. C., Ball, S. F. and Joffrion, V. C. Glaucoma in Hansen's disease. Br. J. Ophthalmol. **75** (1991) 270–272.

Glaucoma is considered to be an uncommon complication of Hansen's disease (leprosy). This study determined the prevalence and characteristics of glaucoma in a large institutionalized leprosy population. All 193 patients currently residing at the Gillis W. Long Hansen's Disease Center, Carville, Louisiana, U.S.A., received a complete ophthalmic examination and review of their records. All had been previously treated with dapsone and/or clofazimine. Based on the strict definition of a recorded intraocular pressure $\geq$ 22 mm Hg and characteristic optic nerve pathology, 19 patients (10%) were found to have glaucoma. Glaucoma secondary to uveitis was noted in 11 of these patients. These findings suggest that the chronic inflammatory process of Hansen's disease, even when treated, may be followed by secondary glaucoma. We recommend that all patients with Hansen's disease receive regular periodic examinations, including slit-lamp biomicroscopy to detect low-grade iridocyclitis and measurement of intraocular pressure.—Authors' Abstract

Zaheer, S. A., Suresh, N. R., Kar, H. K., Sharma, A. K., Mukherjee, A., Mukherjee, R. and Talwar, G. P. Immunological upgrading with combined immunotherapy and chemotherapy in a lepromatous leprosy patient: a case report. Lepr. Rev. **61** (1991) 297–302.

Immunotherapy with *Mycobacterium w* was given, in addition to standard multidrug therapy (MDT) to a lepromatous leprosy (LL) patient with a bacteriological index (BI) of 6. After 15 months of treatment this patient attained bacteriological negativity and clinical inactivity. Histopathologically the patient upgraded to borderline-tuberculoid at 12 months, and at 15 months showed features of nonspecific infiltration in the dermis. The rapid immunological upgrading seen in the patient is highlighted in this paper.—Authors' Summary

Immuno-Pathology

Adams, L. B., Franzblau, S. G., Vavrin, Z., Hibbs, J. B., Jr. and Krahenbuhl, J. L. L-Arginine-dependent macrophage effector functions inhibit metabolic activity of *Mycobacterium leprae.* J. Immunol. **147** (1991) 1642–1646.

Recently, L-arginine has been shown to be a necessary substrate for murine-activated macrophage-mediated tumor cytostasis and microbiostasis of certain fungi, bacteria, and intracellular protozoa. We report here the effects of the L-arginine-dependent pathway of activated mouse macrophages (MØ) on the obligate intracellular prokaryote, *Mycobacterium leprae.* Due to the inability to culture *M. leprae in vitro,* a simple, quantitative assay was employed to measure the metabolism/viability of *M. leprae* released from MØ: the metabolic capacity of *M. leprae* to oxidize ^{14}C-palmitic acid to $^{14}CO_2$. Murine normal MØ or MØ activated *in vitro* with IFN-γ or *in vivo* by injection with *Corynebacterium parvum* were infected with viable *M. leprae* freshly harvested from the foot pads of *nu/nu* mice. Activated MØ strikingly inhibited the metabolism of *M. leprae*; however, in L-arginine-free medium or in medium containing L-arginase, the inhibitory effects of activated MØ on *M. leprae* metabolism were abolished. The competitive inhibitor of L-arginine, N^G-monomethyl-L-arginine, also blocked the inhibitory effects of activated MØ for *M. leprae,* but the addition of supplemental L-arginine overcame the N^G-monomethyl-L-arginine-induced block. Furthermore, in the culture supernatants, the levels of NO_2^-, an end product of L-arginine degradation, were directly proportional to the ability of the activated MØ to inhibit *M. leprae* metabolism. These data present five lines of evidence that suggest that activated MØ utilize the L-arginine-dependent pathway to cope with *M. leprae.*—Authors' Abstract

Chan, J., Fan, X. D., Hunter, S. W., Brennan, P. J. and Bloom, B. R. Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. Infect. Immun. **59** (1991) 1755–1761.

Mycobacterium tuberculosis and *M. leprae,* the causative agents of tuberculosis and leprosy respectively, produce large quantities of lipoarabinomannan (LAM), a highly immunogenic, cell-wall-associated glycolipid. This molecule has been previously reported to be a potent inhibitor of gamma-interferon-mediated activation of murine macrophages. Studies of the mechanism by which this mycobacterial glycolipid down-regulates macrophage effector functions provide evidence that LAM acts at several levels and that it can (i) scavenge potentially cytotoxic oxygen free radicals, (ii) inhibit protein kinase C activity, and (iii) block the transcriptional activation of gamma-interferon-inducible genes in human macrophage-like cell lines. These results suggest that LAM can inhibit macrophage activation and triggering and cytocidal activity and that it may represent a chemically defined virulence factor contributing to the persis-

tence of mycobacteria within mononuclear phagocytes.—Authors' Abstract

Chanteau, S., Cartel, J.-L., Boutin, J.P. and Roux, J. Evaluation of gelatin particle agglutination assay for the detection of anti-PGLI antibodies. Comparison with ELISA method and applicability on a large scale study using blood collected on filter paper. Lepr. Rev. **62** (1991) 255–261.

Given the technical difficulties of the ELISA method, a gelatin particle agglutination test (MLPA) has been developed recently for the detection of anti-PGL-I antibodies. The purpose of this study was to compare these two tests. MLPA was found to be less specific than ELISA (91% versus 98%, $\chi^2 = 66.8$, $p < 0.001$). The sensitivity of both tests was of 95% for the diagnosis of multibacillary patients. In the case of paucibacillary patients, MLPA was found to be less sensitive than ELISA (21% versus 35%, $\chi^2 = 6.98$, $p > 0.01$). The agreement between the two tests for a positive or a negative result was satisfying (85% to 100%), except for the weakly seropositive individuals (71%). The correlation between OD obtained with ELISA and antibody titer obtained with MLPA was statistically significant ($r = 0.70$, $p < 0.001$). Conversely to ELISA, MLPA was not applicable on blood samples absorbed on filter paper without a serious loss of sensitivity. In conclusion, this study demonstrated that the MLPA test can only reliably detect anti-PGL-I antibodies in multibacillary cases.—Authors' Summary

Chukanov, V. I., Litvinov, V. I., Tukhtayev, M. T. and Baensky, A. V. [Antituberculous antibodies in enzyme immunoassay in relation to the efficacy of tuberculosis chemotherapy.] Probl. Tuberk. 7 (1991) 57–59. (in Russian)

A method of indirect solid-phase EIA was used to detect serum antibodies in 110 patients with destructive tuberculosis. The preparation with a molecular mass of 38–42 kDa isolated from the mycobacteria H37Rv was used as an antigen. Changes in the level of antituberculous antibodies were found during treatment, which are most manifested in 4–6 months of antituberculous therapy and depend on treatment efficiency. The effective therapy is accompanied by a decrease in the level of antituberculous antibodies by that time, while in ineffective therapy it remains high. Hence, changes in the level of antituberculous antibodies may serve as an additional criterion of chemotherapy efficiency.—Authors' English Abstract

Cohn, Z. A. and Kaplan, G. Hansen's disease, cell-mediated immunity, and recombinant lymphokines. J. Infect. Dis. **163** (1991) 1195–1200.

Recombinant lymphokines being used in humans are helping us direct and understand the function and interactions of many of the cells that cooperate to generate a cell-mediated immune response. These molecules have potential usefulness as immunotherapeutic agents in the therapy of a number of microbial diseases after careful evaluation of dose route, function, and untoward reactions.—Authors' Conclusions

de Vries, R. R. P. An immunogenetic view of delayed type hypersensitivity. Tubercle **72** (1991) 161–167.

This review, the third in the series on cellular immune reactivity to tubercle bacilli in the centenary year of Koch's classical paper describing this phenomenon and its possible implications, represents an immunogenetic point of view. In fact this will be quite a broad point of view by an immunogeneticist who is not hampered by specific knowledge on therapy or prevention of tuberculosis. In this respect I probably do not differ very much from Robert Koch 100 years ago! An important difference, however, is that we think we now understand a great deal of the cellular and molecular basis of the immunological phenomena observed by Koch. Immunogenetics has contributed considerably to our current understanding and I will try to review that contribution here. Because thus far my main research interest has been in another mycobacterium, namely *Mycobacterium leprae,* I will use *M. leprae* and leprosy as an example to illustrate some ideas. The message of this review is that there is a reason for optimism: the knowledge recently

gained by cellular and molecular immunologists as well as immunogeneticists has straightforward implications for the rational development of subunit vaccines and immunotherapeutic strategies.—Author's Summary

Filley, E. A. and Rook, G. A. W. Effect of mycobacteria on sensitivity to the cytotoxic effects of tumor necrosis factor. Infect. Immun. **59** (1991) 2567–2572.

Unlike *Mycobacterium leprae, M. tuberculosis* is not found inside cells other than macrophages and polymorphonuclear cells *in vivo,* yet previous work has revealed that *in vitro* it readily enters all cell lines tested. Moreover, these cells are not killed by the intracellular mycobacteria. [The authors] report here that when fibroblasts take up live (but not killed) *M. tuberculosis* H37Rv, they develop greatly increased sensitivity to the toxic effects of tumor necrosis factor (TNF) whether the cell line is inherently sensitive to TNF or not. Ultrasonically disrupted *M. tuberculosis* also has this property. The increased sensitivity is seen in the absence of metabolic inhibitors, although addition of emetine, an inhibitor of protein synthesis, causes the effect to manifest itself earlier and at a lower concentration of TNF. In contrast, infection with *M. bovis* bacillus Calmette-Guérin induces little or no increased sensitivity to TNF; whereas *M. avium* and *M. tuberculosis* H37Ra have intermediate sensitivities. [The authors] discuss the possibility that virulent tuberculosis strains produce a factor which distorts the normal protective function of TNF, rendering it toxic to host tissues and leading to the classical immunopathology of tuberculous lesions.—Authors' Abstract

Flesch, I. E. A. and Kaufmann, S. H. E. Mechanisms involved in mycobacterial growth inhibition by gamma interferon-activated bone marrow macrophages: role of reactive nitrogen intermediates. Infect. Immun. **59** (1991) 3213–3218.

Murine bone-marrow derived macrophages are able to inhibit the growth of *Mycobacterium bovis* after stimulation with recombinant gamma-interferon. This antimycobacterial activity was inhibited by N^G-monomethyl-L-arginine, a specific inhibitor of nitrite and nitrate synthesis from L-arginine. Furthermore, there was a complete lack of mycobacterial growth inhibition in a medium deficient in L-arginine. Nitrite is generated by gamma-interferon-activated bone-marrow-derived macrophages after infection with *M. bovis,* and a correlation between mycobacterial growth inhibition and nitrite production was observed. These results indicate that reactive nitrogen intermediates derived from L-arginine are crucially involved in macrophage antimycobacterial activity.—Authors' Abstract

Foss, N. T., Pagnano, P. M. G., Bechelli, L. M. and Lima Filho, E. C. Blastogenic response with autologous plasma and Mitsuda's reaction in leprosy patients and their unaffected sibs. Acta Leprol. (Genève) **7** (1991) 335–345.

Many investigators have evaluated the influence of genetic constitution on the susceptibility to leprosy in studies linked to different types of research. To determine the possible existence of a family trait linked to the lymphoproliferation and to lepromin reactivity we studied the blastogenic response to phytohemagglutinin, lepromin and *Mycobacterium leprae* and the Mitsuda reaction in leprosy patients and their unaffected sibs. Sixty-eight individuals were studied, 34 were leprosy patients (17 lepromatous and 17 tuberculoid leprosy) and the remaining were their sibs previously matched by sex and age. The indices of blastogenesis and lepromin reactivity were lower in lepromatous than in tuberculoid patients, that confirmed the immunological polarity of the two types of leprosy. Both the lymphoproliferation and Mitsuda reaction results suggest different cell-immune responses in leprosy patients and their unaffected sibs, so that the hypothesis of a family trait favoring the similarity of responses to these tests among sibs becomes unlikely.—Authors' Summary

Fournie, J. J., Mullins, R. J. and Basten, A. Isolation and structural characteristics of monoclonal antibody-defined cross-reactive phospholipid antigen from *Mycobacterium tuberculosis* and *Mycobacteri-*

um leprae. J. Biol. Chem. **266** (1991) 1211–1219.

A monoclonal antibody (L4) originally raised against an extract of *Mycobacterium leprae* has been further characterized and shown to be specific for mycobacterial phosphatidyl inositol mannosides (PIM). Antibody-reactive material isolated from *M. tuberculosis* was identified by gas chromatography–mass spectroscopy as phosphatidyl inositol dimannoside with further studies showing that the antibody also recognized the related pentamannoside version of the glycolipid. Epitope analysis identified a key role for the axial 2-hydroxyl of the alpha-mannoside residues in monoclonal antibody binding without detectable contribution from the myo-inositol. Antibodies to PIM were detected in sera from patients with mycobacterial disease; a potential role for PIM as a serodiagnostic antigen is discussed.—D. Young (Trop. Dis. Bull.)

Gatrill, A. J., Munk, M. E. and Kaufmann, S. H. E. Gamma/Delta T cells and bacteria. Res. Immunol. **141** (1990) 641–644.

The findings described show that γ/δ T cells can perform both of the principal T-cell functions, namely, interleukin secretion and target cell lysis. However, our findings do not indicate whether the two functions are associated with distinct subpopulations or whether bifunctional γ/δ T cells exist. Thus, functionally, bacteria-reactive γ/δ T cells do not principally differ from their counterparts expressing the α/β TcR. Perhaps the major differences in these two populations lie in differential activation requirements, genetic restriction, tissue distribution and antigen recognition. Our data using killer assays with γ/δ T cells suggest antigen-specific recognition of bacterial ligands. In addition, there may still be some broader activation through mitogenic or superantigen-like components. Superantigen stimulation of γ/δ T cells by staphylococcal enterotoxins has been reported (Rust *et al.*, 1990). We found that *Mycobacterium tuberculosis*-activated γ/δ T cells proliferated upon restimulation in response to a variety of bacteria. Also, we see NK activity (lysis of K562 cells) with many, though not all, *M. tuberculosis*-activated γ/δ T cells. In summary, we believe that our findings provide a small bit of information toward a better understanding of γ/δ T-cell nominal antigen recognition and function. They further challenge us to pursue the question of whether γ/δ T cells have a function in immunity to bacteria. *In situ* studies indicating accumulation of γ/δ T cells in reversal reactions of leprosy patients and in and around necrotic lesions of tuberculous lymphadenitis are consistent with such an idea. However, we have to admit that, up to now, we have not found increased numbers of γ/δ T cells in lung lavages of tuberculosis patients. If γ/δ T cells do prove to have a role, this might not be restricted to mycobacteria, nor even to intracellular bacteria, but may be extended to combatting various bacterial infections.—Authors' Conclusions

Haanen, J. B. A. G., Malefijt, R. de W., Res, P. C. M., Kraakman, E. M., Ottenhoff, T. H. M., de Vries, R. R. P. and Spits, H. Selection of a human T helper type 1-like T cell subset by mycobacteria. J. Exp. Med. **174** (1991) 583–592.

Mycobacteria elicit a cellular immune response in their hosts. This response usually leads to protective immunity, but may sometimes be accompanied by immunopathology due to delayed-type hypersensitivity (DTH). A striking example in man is tuberculoid leprosy, which is characterized by high cellular immunity to *Mycobacterium leprae* and immunopathology due to DTH. Skin lesions of patients suffering from this disease have the characteristics of DTH reactions in which macrophages and CD4+ T lymphocytes predominate. In animal models, it has been shown that DTH responses are associated with the presence of a particular subset of CD4+ T cells (T helper type 1 [Th1]) that secrete only certain cytokines, such as interleukin 2 (IL-2), interferon γ (IFN-γ), and lymphotoxin, but no IL-4 or IL-5. We studied the cytokine release of activated *M. leprae*-reactive CD4+ T-cell clones derived from tuberculoid leprosy patients. These T-cell clones, which were reactive with mycobacterial heat-shock proteins, exhibited a Th1-like cytokine secretion pattern with very high levels of IFN-γ. Half of these clones se-

creted low levels of IL-4 and IL-5, but the ratio of IFN-γ to IL-4 and IL-5 was much higher than that of T-cell clones reactive with nonmycobacterial antigens. A Th1-like cytokine secretion pattern was also observed for T-cell clones and polyclonal T-cell lines from control individuals that recognized both heat-shock and other mycobacterial antigens. The levels of IFN-γ secreted by these clones were, however, significantly less than those of patient-derived T-cell clones. This Th1-like pattern was not found with T-cell clones from the same patients and healthy individuals generated in the same manner, but reactive with nonmycobacterial antigens. Our data thus indicate that mycobacteria selectively induce human T cells with a Th1-like cytokine secretion profile.—Authors' Summary

Kaplan, G., Britton, W. J., Hancock, G. E., Theuvenet, W. J., Smith, K. A., Job, C. K., Roche, P. W., Molloy, A., Burkhardt, R., Barker, J., Pradhan, H. M. and Cohn, Z. A. The systemic influence of recombinant interleukin 2 on the manifestations of lepromatous leprosy. J. Exp. Med. **173** (1991) 993–1006.

Fourteen patients with lepromatous leprosy received twice daily injections of 10 μg recombinant interleukin 2 (rIL-2), by the intradermal route, in the skin of the back for 8 d (total dose, 160 μg). Lymphokine administration was accomplished without drug toxicity or the development of acute nerve damage. The majority of patients developed nontender axillary lymphadenopathy during the course of treatment. Local injection sites showed progressively larger zones of induration, peaking at 24 hr and persisting for many days. Early 12-hr reactions were of a macular, erythematous nature and exhibited an increasingly striking diurnal variation. The morning injection sites were three- to fourfold larger in diameter than those placed in the evening (9 am to 9 pm). Systemic manifestations of intradermal rIL-2 administration were noted. Peripheral blood T cells, including CD4+ and CD8+ phenotypes, increased 2–2.5-fold and NK cells increased sixfold. Elevations in [^{3}H]TdR incorporation into peripheral blood mononuclear cells occurred to a variety of mycobacterial antigens, but not to those of *Mycobacterium leprae*. Within 2 wk, biopsies at sites far removed from the back showed increased infiltration of mononuclear cells in 12 of 14 patients. Immunocytochemistry revealed the presence of newly emigrated CD4+ T cells, monocytes, and dermal CD1+ Langerhans' cells. Endothelial cells of small dermal vessels expressed major histocompatibility complex class II determinants on their surface. Transmission electron microscopy of these specimens revealed markedly enlarged endothelial cells with many surface projections extending into the lumen as well as extravasating lymphoid cells. The numbers of acid-fast *M. leprae* in the peripheral sites were examined by slit smear and in biopsies of matched leprosy lesions taken before and after IL-2 administration. Within 2 mo, slit smears showed a 0.5 log or greater reduction in 12 of 14 patients, with a mean for all patients tested of 0.5 log units. Biopsy specimens showed a 1 log unit or greater reduction in the bacterial index (BI) in 6 of 14 patients. Historical controls in this Nepalese population showed a 0.5 log unit reduction after multidrug therapy over a period of 12 mo. Thus, after 8 d of IL-2 injections, a fivefold reduction in BI was observed during the first 2 mo of the study. Antibody levels against *M. leprae* phenolic glycolipid-I (PGL-I) and lipoarabinomannan B were markedly elevated after IL-2 injections, while PGL-I antigen levels were reduced. We conclude that the administration of rIL-2 has had a significant effect in decreasing the total body burden of *M. leprae*. This is accomplished by the influx of mononuclear leukocytes from the circulation, the enhancement of cell-mediated immunity, and the degradation of leprosy bacilli. This occurs much more rapidly compared with multidrug chemotherapy alone.—Authors' Abstract

Kaplan, G. and Cohn, Z. A. Leprosy and cell-mediated immunity. Curr. Opin. Immunol. **3** (1991) 91–96.

The intradermal injection of the purified protein derivative of tuberculin into lepromatous leprosy patients leads to a local cell-mediated immune response and to the extensive destruction of *Mycobacterium leprae*. This local response also occurs after

intradermal injection of recombinant human interleukin-2; when administered over an 8-day period interleukin-2 evokes a systemic cell-mediated immune response and a reduction in the bacillary burden.—Authors' Abstract

Kohli, M., Ganguly, N. K., Kaur, S., Chugh, K. S., Sharma, V. K. and Bhushnurmath, S. R. Does Convit vaccine (BCG + *Mycobacterium leprae*) afford protection against biochemical changes in renal brush border membrane in experimental leprosy? Lepr. Rev. **62** (1991) 269–275.

Renal functional status in *Mycobacterium leprae*-infected mice can be best studied by examining the enzymatic status of brush-border membrane vesicles from proximal convoluted tubule. The role of vaccination in modulation of the renal status brought by the disease has been studied using this technique. The characteristic marker enzymes of renal brush-border membrane—namely, alkaline phosphatase, leucine aminopeptidase, and γ-glutamyl transpeptidase decreased significantly ($p < 0.01$) in due course in *M. leprae* infection over a period of 9 months. The combined vaccine (BCG + *M. leprae*) may have a protective effect on renal abnormalities only in the initial stages of infection as indicated by a significant rise in enzymatic levels. However, no significant ($p > 0.05$) protective effect of vaccine was found in a more advanced disease state after 9 months in infected mice.—Authors' Summary

Li, X.-Y., *et al.* [A preliminary analysis of antigen components of *M. leprae* with SDS-PAGE.] China Lepr. J. 7 (1991) 147–150. (in Chinese)

A preliminary study has been conducted on antigenic analysis of *Mycobacterium leprae* (ML) derived from infected armadillos, *M. avium, M. marinum, M. smegmatis, M. scrofulaceum* and BCG. Firstly, the supernatants of 6 bacterial sonic extracts were fractionated by SDS-PAGE using 15% polyacrylamide gel. Subsequently, the proteins in the gel were stained with Coomassie brilliant blue and the separated protein bands from mycobacteria were obtained. The results indicated that the spectra of the antigen bands from 6 mycobacteria were different. The number of antigen bands in ML is only half of the other 5 species of mycobacteria, displaying 20 protein bands in which 14.5 kDa, 16.5 kDa, 20 kDa, 22 kDa, 25 kDa, 40 kDa, 52 kDa, 60 kDa and 73 kDa bands are the major components; 50 kDa protein band in *M. avium,* 31 kDa band in *M. smegmatis* and 24.5 kDa band in BCG are more marked. The authors think that the spectrum of antigen bands has an important and potential significance for distinguishing and identifying species of mycobacteria and preparing effective absorbent reagents for serodiagnostic tests, and even it favored finding new antigens which could play an important role in immunologic reactions for leprosy. Relevant techniques for sensitivity, stability and reproducibility of SDS-PAGE are discussed in detail.—Authors' English Abstract

Longo, I. M., Moura, N. C., Opromolla, D. and Mendes, N. F. Quantitation of the soluble E-receptor of human T lymphocytes by rocket electrophoresis in the serum of patients with lepromatous and tuberculoid leprosy. Medicina (B. Aires) **51** (1991) 133–136.

Human T lymphocytes carry a membrane receptor for sheep erythrocytes (E) which is responsible for the well-known phenomenon of E-rosette formation. This receptor has been related to CD2 molecules; it is present in a soluble form (Rs) in normal serum and may play an immunoregulatory role. In this study we quantitated soluble E-receptor in serum samples of 43 normal controls, 32 patients with tuberculoid leprosy, and 53 with lepromatous leprosy, using rocket electrophoresis and an anti-E-receptor serum (anti-Rs) obtained from an adult sheep immunized with autologous E treated with Rs. In the three groups studied, the rocket means were respectively 5.0, 7.5 and 10.9 mm ($p < 0.001$). We found abnormally high levels of Rs in the serum of various diseases associated with a depression of cell-mediated immunity. The increase of Rs levels in the serum may be one of the mechanisms responsible for the depression of cellular immunity in leprosy.—Authors' Summary

McKenzie, K. R., Adams, E., Britton, W. J., Garsia, R. J. and Basten, A. Sequence and immunogenicity of the 70-kDa heat shock protein of *M. leprae*. J. Immunol. **147** (1991) 312–319.

The gene encoding the *Mycobacterium leprae* 70-kDa heat-shock protein (hsp70) has been isolated from a cosmid library using a fragment of the clone JKL2. Southern blot analysis of a positive clone identified a 4.4-kb fragment containing the entire coding region of the gene plus 2.4 kb upstream. Sequencing revealed the gene to encode a 621-amino-acid protein, bearing 56% identity with the *Escherichia coli dna*K gene product and 47% and 46% identity with the human and *Caenorhabditis elegans* hsp70, respectively. Comparison with the C-terminal 203 amino acids of the *M. tuberculosis* 71-kDa antigen yielded 70% identity. Recombinant *M. leprae* p70 was produced in *E. coli* as a fusion protein (rp70f) with a portion of the schistosomal glutathione-S-transferase, using the expression vector, pGEX-2T. Cleavage with thrombin resulted in the release of a 70.0-kDa protein (rp70c) from the glutathione-S-transferase. Examination of the proteins by immunoblotting demonstrated that anti-*M. leprae* monoclonal antibody (mAB), L7, and sera from lepromatous leprosy patients bound to both the cleaved and fusion proteins. We compared the T-cell reactivity of the *M. leprae* recombinant proteins with that of mAb affinity-purified bacille Calmette-Guérin (BCG) 70-kDa antigen using proliferation assays. PBMC of BCG vaccinees responded to both *M. leprae* cleaved and fusion p70, though more subjects responded to the rp70c (18 of 20) than to rp70f (13 of 20). Responses were generally higher to rp70c than to rp70f, however all responses to the *M. leprae* recombinant proteins were lower than to mAb affinity-purified BCG p70. Thus, the *M. leprae* 70-kDa heat-shock protein elicits T- and B-cell responses in subjects exposed to mycobacteria, despite its homology with the human hsp70.—Authors' Abstract

Meng, M.-B., *et al.* [Evaluation of MLPA for determination of antibody to PG-1 of MB leprosy.] China Lepr. J. **7** (1991) 81–85. (in Chinese)

The comparison of MLPA with ELISA, using PG-I as antigen, proved that the MLPA has quite high sensitivity and specificity as compared with ELISA. The concordance rate between MLPA and ELISA is 90.5%. Therefore, the authors think that the kit with reagents of MLPA could be extensively used, and that the limit of positivity should be generally determined at 1:32 $\geq \pm 3$, but it should be at 1:16 $\geq \pm 3$ in large scale so as to simplify the process and save reagents.—Authors' English Abstract

Miko, T. L. and Lucas, S. B. Acid-fast and H & E stainings can be combined better than in the TRIFF method. (Letter) Lepr. Rev. **61** (1990) 396–398.

The correspondents present details of a combined hematoxylin and eosin acid-fast stain suitable for staining sections of tissue. The method is simple and reliable and of value when only a single section is available for the diagnosis of leprae. Compared with the alternative TRIFF staining method, the main advantage appears to be the elimination of 5 alcohol steps during staining thus reducing the chance of overdecolorization of bacilli.—B. W. Allen (Trop. Dis. Bull.)

Pessolani, M. C. V., Peralta, J. M., Rumjanek, F. D., Gomes, H. M., Marques, M. A. de M., Almeida, E. C. C., Saad, M. H. F. and Sarno, E. N. Serology and leprosy: immunoassays comparing immunoglobulin G antibody responses to 28- and 30-kilodalton proteins purified from *Mycobacterium bovis* BCG. J. Clin. Microbiol. **29** (1991) 2285–2290.

Two major proteins from *Mycobacterium bovis* BCG culture filtrates with molecular masses of 28 kDa (P_{28}) and 30 kDa (P_{30}), identified as components of the BCG 85 complex, were purified and used in enzyme-linked immunosorbent assays (ELISAs) for the determination of specific immunoglobulin G (IgG) levels in patients with leprosy or tuberculosis or with exposure to these diseases. High reactivity to both antigens was observed with sera from lepromatous leprosy patients, whereas antibody levels in sera from paucibacillary leprosy patients were not significantly different from those in sera from healthy individuals from an

area in which leprosy is endemic. High IgG responses were also found in some contacts of lepromatous leprosy patients. A comparison of the levels of anti-P_{28} and anti-P_{30} within the multibacillary leprosy patient group showed much higher IgG reactivity to P_{28} than to P_{30}, suggesting that the antibody response of lepromatous patients is directed predominantly against the 28-kDa protein. A high degree of correlation in values of ELISAs based on P_{28} and on the phenolic glycolipid of *M. leprae* was observed in all groups analyzed. The potential use of an assay based on the 28-kDa protein to selectively distinguish individuals destined to develop multibacillary leprosy is discussed, as also is the likelihood that the 28-kDa–30-kDa complex, part of the fibronectin-binding family, is an important component of *M. leprae*.—Authors' Abstract

Ramasesh, N., Adams, L. B., Franzblau, S. G. and Krahenbuhl, J. L. Effects of activated macrophages on *Mycobacterium leprae*. Infect. Immun. **59** (1991) 2864–2869.

Five alternative methods were used to explore *in vitro* the effects of normal and activated murine macrophages on the metabolic well-being of intracellular *Mycobacterium leprae*: fluorescein diacetate-ethidium bromide staining, ATP content, synthesis of phenolic glycolipid I, and two techniques to quantitate oxidation of palmitic acid. In relatively short-term experiments (7 to 10 days), each of these procedures provided strong evidence that activated macrophages exerted a deleterious effect on the leprosy bacillus. These findings appear to confirm the contention that activated macrophages underlie host resistance to clinical leprosy and limitation of *M. leprae* growth in paucibacillary leprosy.—Authors' Abstract

Rambukkana, A., Yong, S. and Das, P. K. Identification of a novel B-cell epitope of restricted specificity on the hsp 65-kDa protein of *Mycobacterium tuberculosis*. FEMS Microbiol. Immunol. **76** (1991) 39–46.

A B-cell epitope on the carboxy-terminal region of the mycobacterial 65-kDa heat-shock protein that distinguishes *Mycobacterium tuberculosis/M. bovis* BCG from *M. leprae* was identified by two novel monoclonal antibodies (mAbs), N35 and Nd4. These mAbs also showed a limited cross-reactivity with mycobacterial species belonging to *M. tuberculosis* complex and *M. avium* complex with the exception of *M. vaccae*. Characterization of the epitope recognized by these mAbs was done with *M. bovis* BCG 65-kDa fusion proteins expressed in *Escherichia coli* encoding various segments of the 65-kDa protein. Our results together with those reported in the literature indicated that this epitope resides in the highly divergent region of amino acid residues 525 to 540. This B-cell epitope on the 65-kDa protein of *M. tuberculosis/M. bovis* BCG has not been recognized by previously reported mAbs, although the analogous epitope sequence of *M. leprae* 65-kDa has been identified by a known mAb (IIIC8) reported in the literature. Therefore the Ne5/Nd4 epitope could be considered important in studying the differential immune response of the host against infections with *M. tuberculosis* complex/*M. avium* complex and *M. leprae*.—Authors' Summary

Salgame, P., Abrams, J. S., Clayberger, C., Goldstein, H., Convit, J., Modlin, R. L. and Bloom, B. R. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. Science **254** (1991) 279–282.

Functional subsets of human T cells were delineated by analyzing patterns of lymphokines produced by clones from individuals with leprosy and by T-cell clones of known function. CD4 clones from individuals with strong cell-mediated immunity produced predominantly interferon-γ; whereas those clones that enhanced antibody formation produced interleukin-4. CD8 cytotoxic T cells secreted interferon-γ. Interleukin-4 was produced by CD8 T-suppressor clones from immunologically unresponsive individuals with leprosy and was found to be necessary for suppression *in vitro*. Both the classic reciprocal relation between antibody formation and cell-mediated immunity and resistance or susceptibility to certain infections may be explained by T-cell subsets differing in patterns

of lymphokine production.—Authors' Abstract

Schurr, E., Malo, D., Radzioch, D., Buschman, E., Morgan, K., Gros, P. and Skamene, E. Genetic control of innate resistance to mycobacterial infections. Immunol. Today **12** (1991) A42–A45.

The genetically determined defect in *Bcg*^{s} macrophage activation and mycobacterial activity could possibly occur at a number of points in the series of biochemical reactions that connect the membrane perturbation induced by the invading mycobacteria with the emergence of antimycobacterial effector mechanisms. At least two general models explaining the single gene effect of *Bcg* on macrophage activation may be considered. First, the two alleles of the *Bcg* gene may encode a functional wild-type and nonfunctional mutant form of a (structural) molecule directly involved in signal transduction. Dysregulation of diacylglycerol-inositol triphosphate metabolism, protein kinase activation or production of secondary messengers such as cAMP may occur in the mutant. Second, the *Bcg* gene may encode a DNA-binding protein that controls transcriptional events associated with macrophage activation. In this context, it is interesting to note that IFN-γ treatment enhances the pleiotropic effects of the *Bcg* gene, which possibly reflects an interaction of the *Bcg* gene product with IFN-responsive sequences.—Authors' Conclusion

Sehgal, V. N., Bhattacharya, S. N., Shah, Y., Sharma, V. K. and Gupta, C. K. Soluble interleukin-2 receptors: levels in leprosy, and during and after type 1 (lepra) and type 2 (ENL) reactions. Lepr. Rev. **62** (1991) 262–268.

Twenty-five patients with type 1 (lepra) and type 2 (ENL) reactions were assayed for SIL-2R in serum—before and after treatment for their acute condition—and the results were compared with 10 normal healthy adults and 20 patients of leprosy *per se*. Classification of each subject into different leprosy groups, and into various types and subtypes of reactions, was done according to standard criteria, prior to inclusion into the study. Detailed statistical evaluation of the data revealed significantly higher levels of SIL-2R in all leprosy patients, as compared to normal controls, with higher levels in the multibacillary groups as compared to the paucibacillary group. SIL-2Rs appeared higher in type 1 upgrading reaction than in other forms of reaction, though this was not statistically significant. There was no significant change in levels following treatment and clinical remission.—Authors' Summary

Sehgal, V. N., Chaudhry, A., Sharma, V. K. and Gupta, C. K. Characterization of circulating lymphocytes by monoclonal antibodies in childhood and adult leprosy. Int. J. Dermatol. **30** (1991) 780–784.

Peripheral blood lymphocyte assays using monoclonal antibodies were done in 66 patients with leprosy, consisting of 25 children and 41 adults. The results were statistically analyzed for correlations, if any, among the different age groups and matched controls. The results, however, failed to show any significant correlation, nor was it possible to draw any conclusion as to why the disease spectrum in children tends to be incomplete (i.e., there is a low incidence of the highly bacilliferous form of disease expression).—Authors' Abstract

Sen, P., Sehgal, P. K., Dixit, V., Singh, U., Chaudhary, S. D., Sikka, R. and Jain, V. K. Lipid-laden macrophages in bone marrow of leprosy patients. Lepr. Rev. **62** (1991) 374–380.

While conducting a study to observe bone-marrow cytomorphological changes in multibacillary (MB) leprosy, lipid-laden macrophages as seen in sphingolipidoses were noted. The present study was planned to observe the occurrence and morphological characterization of these macrophages in various types of leprosy. Bone-marrow records from 48 cases of paucibacillary (PB) and 72 cases of MB leprosy were analyzed. The macrophages accounting at the most for 3.5% of marrow cells were observed in 5 cases of PB and 43 cases of MB leprosy with a maximum incidence being observed in patients with erythema nodosum leprosum (ENL) (16/17). The lipid present in the cytoplasm of these cells could be derived from

the lipid of the cell wall of *Mycobacterium leprae.* To the best of our knowledge, these cells have not been reported in leprosy so far.—Authors' Summary

Shu, H.-W., *et al.* [A study of subclinical infection with *M. leprae,* the factors influencing the subclinical infection.] China Lepr. J. **7** (1991) 143–147. (in Chinese)

The results of detecting antibodies against PG-1 with ELISA in 452 household contacts of leprosy patients are reported and possible factors influencing upon subclinical infection with *Mycobacterium leprae* (SI) are analyzed. The positive rate of SI is 34.84% in contacts with LL, 17.11% in those with BT, 32.11% in those with active patients, and 24.44% in those with inactive ones, suggesting that the degree of exposure to *M. leprae,* i.e., the number and viability of *M. leprae* contacted, is one of the most important factors for the positivity. The higher SI rate was seen in the 3rd and 4th years after the exposure to leprosy patients, but the distribution of yearly positivity is not regular. The authors regard this as the result of deviation in calculating the duration contacted both subjectively and objectively. Effect of blood relationship between the contacts and the patients on the positive rate of SI is obviously seen, i.e., the rate is 50.0% in leprosy patient's children and 27.97% in the patient's spouse, suggesting that long-term contact with the patients is also an important factor for the SI. Out of 23 children of parents suffering from leprosy, both MB or one MB and the other PB, 11 children (47.8%) were positive, but all of 11 children of PB leprosy parents were negative, suggesting that the SI might be related to resistance, immunity and susceptivity of the contacts themselves. The effects of age and sex on the SI are described.—Authors' English Abstract

Steinhoff, U., Schoel, B. and Kaufmann, S. H. E. Lysis of interferon-γ activated Schwann cell by cross-reactive CD8+ α/β T cells with specificity for the mycobacterial 65 kd heat shock protein. Int. Immunol. **2** (1989) 279–284.

Heat-shock protein (hsp) 65 is a major T-cell antigen of *Mycobacterium leprae.* The hsp65 of *M. leprae* is nearly identical in *M. bovis/M. tuberculosis* (> 95% protein sequence homology) and surprisingly similar in man (65% protein sequence homology). Recently, we had provided evidence in a murine model that CD8+ T cells recognize and lyse Schwann cells presenting *M. leprae* antigen in the context of major histocompatibility (MHC) class I gene products. Because murine Schwann cells are class I negative, antigen presentation requires prior stimulation with interferon-γ (IFN-γ). CD8+ T cells were activated against tryptic fragments of mycobacterial hsp65. These T cells recognized epitopes of hsp65 which had been generated through the cytoplasmic class I processing pathway. They were also capable of lysing Schwann cells which had been activated by IFN-γ and not primed with nominal hsp65 peptides. In contrast, T cells activated against tryptic ova peptides only lysed Schwann cells which had been both stimulated with IFN-γ and primed with ova peptides. Evidence is presented that class I (H-2D) restricted, CD8+ α/β T lymphocytes with specificity for the mycobacterial hsp65 recognize IFN-γ-stimulated Schwann cells probably because they are specific for a(n) epitope(s) shared by the bacterial hsp and a host cognate. Activation of autoreactive T cells with specificity to shared epitopes could contribute to nerve damage in tuberculoid leprosy which is characterized by low to absent *M. leprae* in Schwann cells.—Authors' Abstract

Tsuyuguchi, I., Kawasumi, H., Ueta, C., Yano, I. and Kishimoto, S. Increase of T-cell receptor γ/δ-bearing T cells in cord blood of newborn babies obtained by in vitro stimulation with mycobacterial cord factor. Infect. Immun. **59** (1991) 3053–3059.

Cord blood T lymphocytes proliferated *in vitro* in response to mycobacterial organisms but did not proliferate in the presence of tuberculin purified protein derivative. Components recognized by cord blood T cells were resistant to protease digestion. In contrast, T lymphocytes derived from tuberculin-positive adult peripheral blood proliferated when stimulated by the protease-sensitive component of mycobacterial

organisms or purified protein derivative, confirming that adult T cells respond to protein components whereas cord blood T cells respond to the nonpeptide component of mycobacteria. *In vitro* culture of cord blood lymphocytes stimulated by either mycobacterial lysates or the lipid fraction showed increases in the numbers of T-cell receptor (TcR) γ/δ T lymphocytes with no changes in the number of TcR α/β T lymphocytes in contrast to the *in vitro* cultures of adult blood lymphocytes stimulated with mycobacterial ligands in which no increase of TcR γ/δ cells was observed. Interleukin-2 receptor (CD25) and Ia antigen (HLA-DR) analyses evidenced the activation of a large proportion of cord blood γ/δ T cells which had increased after stimulation with mycobacteria *in vitro*. Further characterization of mycobacterial ligand suggested that the lipid fraction of mycobacterial lysate or trehalose dimycolate-cord factor was the most plausible cause for T-cell proliferation in cord blood. These results suggest that when the γ/δ T cells in a newborn infant not yet sensitized to any pathogenic organisms are confronted by a mycobacterium, they respond nonspecifically to the mycobacterial organism or its lipid component (cord factor). γ/δ T cells may therefore play a distinct role in forming the first line of the host defense system against certain microorganisms.—Authors' Abstract

Uyemura, K., Deans, R. J., Band, H., Ohmen, J., Panchamoorthy, G., Morita, C. T., Rea, T. H. and Modlin, R. L. Evidence for clonal selection of γ/δ T cells in response to a human pathogen. J. Exp. Med. **174** (1991) 683–692.

T cells bearing γ/δ antigen receptors comprise a resident population of intraepithelial lymphocytes in organs such as skin, gut, and lungs, where they are strategically located to contribute to the initial defense against infection. An important unsolved question about antigen-driven γ/δ T cell responses regards the breadth of their T cell receptor (TCR) repertoire, since many specific epithelial compartments in mice display limited diversity. [The authors] have examined the diversity of TCR δ gene expression among human γ/δ T cells from skin lesions induced by intradermal challenge with *Mycobacterium leprae*. [The authors] show that the vast majority of γ/δ cells from *M. leprae* lesions use either Vδ1-Jδ1 or Vδ2-Jδ1 gene rearrangements and, within a given region of the lesion, display limited junctional diversity. This contrasts markedly with the extensive diversity of γ/δ T cells from peripheral blood of these same individuals, as well as skin from normal donors. These results indicate that the γ/δ response to *M. leprae* involves the selection of a limited number of clones from among a diverse repertoire, probably in response to specific mycobacterial and/or host antigens.—AS (Trop. Dis. Bull.)

Vemuri, N. and Mukherjee, R. Immunoreactivity of nerve lipid antigens in leprosy. J. Cin. Lab. Analysis **5** (1991) 157–161.

Neural lipid antigens, namely, galactocerebroside and ganglioside, have been implicated in demyelinating diseases. We were interested in finding the role of these antigens in leprosy neuritis. The humoral immune response to these lipid antigens was quantitated by enzyme-linked immunosorbent assay in sera from 91 leprosy patients and 18 normal individuals. Our data revealed the presence of antibodies to total nerve lipids (TNL) and galactocerebroside (GalC) and a significantly low level to ganglioside (Gg) in all the categories of leprosy. No antilipid antibodies were detected in normals. Anti-TNL and anti-GalC antibodies were highest in tuberculoid leprosy patients. Statistically significant positive correlation was observed between anti-TNL and anti-GalC antibodies in lepromatous borderline, tuberculoid, and neuritic patients.—Authors' Abstract

Wu, Q.-X., *et al.* [A comparison of serological activities of synthetic leprosy antigens—F/NT, ND and NT.] China Lepr. J. **7** (1991) 138–143. (in Chinese)

A comparison of serological activity of synthetic antigens F/NT, ND and NT has been completed with using ELISA in patients with leprosy (103 cases: LL 25, BL 25, BB 3, BT 26 and TT 24) and tuberculosis (100 cases), and normal controls (NC 100). The results indicated that in sera of

TB and NC, all ELISAs are negative by three practical normal values (PNV 1 < PNV II < PNV III), but in leprosy patients' sera, even if by PNV III, the positive rates in all ELISAs are 88.3% to 92.4% on the average, and there is no difference among the positivity rates of the three kinds of antigens (u value = 0.958 ~ 1.330 < 1.96, $p > 0.05$), and the individual agreement (IA) is more than 93.2%, indicating that the three ELISAs are highly comparable and that rank correlation analyses (Spearman's method) of the three ELISAs showed significant correlation between their OD value in every ELISA tested and BI in leprosy patients (r, ND = 0.875, $p < 0.05$; r, NT = 0.893, $p < 0.05$; r, F/NT = 0.929, $p = 0.01$), and in the coordinate the increase of OD value is associated with increase of BI, almost approximating linearization. The evaluation of the three ELISAs according to parameters obtained from the tests indicated that, even if by PNV III, the three ELISAs are highly sensitive and specific for detecting infection with *Mycobacterium leprae,* their positive and negative prediction values are more than 0.9, and their Youden's index is more than 0.8 as well. If by PNV I, all of the parameters above mentioned would be more than 0.9. The authors consider that F/NT is a valuable antigen, and besides its practicality as those of ND and NT its economic efficacy is higher.—Authors' English Abstract

Yadava, A., Suresh, N. R., Zaheer, S. A., Talwar G. P. and Mukherjee, R. T-cell responses to fractionated antigens of *Mycobacterium w,* a candidate anti-leprosy vaccine, in leprosy patients. Scand. J. Immunol. **34** (1991) 23–31.

Mycobacterium w, an atypical cultivable mycobacterium, is undergoing phase III clinical trials as a vaccine against leprosy in India. It has brought about lepromin conversion and histopathological upgradation in a significant number of patients studied so far. It is important to identify antigens of *M. w* that trigger T-cell responses in leprosy patients vaccinated with this organism. In the present study the peripheral T-cell repertoire of 12 *M. w*-vaccinated leprosy patients, 10 unimmunized leprosy patients, 8 tuberculoid and 5 healthy contacts was analyzed with fractionated antigens of *M. w.* The lepromatous leprosy patients who are in general anergic to antigens of *M. leprae* did not respond to antigens of *M. w.* However, peripheral blood mononuclear cells obtained from leprosy patients who had been vaccinated with *M. w* responded to many antigens. These responses were frequently directed against low molecular weight entities of 14–45 kDa. T cells from tuberculoid leprosy patients and healthy contacts also responded predominantly to a number of low molecular weight antigens of *M. w.* The study also identified an immunodominant 28–31 kDa antigenic fraction carrying T- as well as B-cell activating determinants.—Authors' Abstract

Yamamura, M., Uyemura, K., Deans, R. J., Weinberg, K., Rea, T. H., Bloom, B. R. and Modlin, R. L. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science **254** (1991) 277–279.

The immunological mechanisms required to engender resistance have been defined in few infectious diseases of man, and the role of specific cytokines is unclear. Leprosy presents clinically as a spectrum in which resistance correlates with cell-mediated immunity to the pathogen. To assess *in situ* cytokine patterns, messenger RNA extracted from leprosy skin-biopsy specimens was amplified by the polymerase chain reaction with 14 cytokine-specific primers. In lesions of the resistant form of the disease, messenger RNAs coding for interleukin-2 and interferon-γ were most evident. In contrast, messenger RNAs for interleukin-4, interleukin-5, and interleukin-10 predominated in the multibacillary form. Thus, resistance and susceptibility were correlated with distinct patterns of cytokine production.—Authors' Abstract

Zumla, A., Williams, W., Mudd, D., Locniskar, M., Behrens, R., Isenberg, D. and McAdam, K. P. W. J. Expression of a common idiotype PR4 in the sera of patients with leprosy. Clin. Exp. Immunol. **84** (1991) 522–526.

The sera of 187 patients from across the leprosy spectrum were screened for the expression of the PR4 idiotype, which was first

identified on a human hybridoma-derived monoclonal antibody from a patient with leprosy and found to react with the *Mycobacterium leprae* phenolic glycolipid and a variety of polynucleotides. Sixty percent (51 out of 85) of patients with lepromatous leprosy (LL), 66% (33 out of 49) with borderline lepromatous (BL) disease, 47% (14 out of 30) with borderline tuberculoid (BT) leprosy, and 56% (13 out of 23) of tuberculoid (TT) patients were found to have significantly elevated titers of the PR4 idiotype in their sera compared with endemic controls, irrespective of the presence or absence of endemic malaria. Sera from 52 patients with tuberculosis were also screened as a control for mycobacterial infection. The PR4 idiotype was significantly elevated in 37% (19 out of 52) of these patients. No correlation between idiotype and serum immunoglobulins IgG and IgM was found, indicating that the concentrations of idiotype levels in sera were not merely a reflection of changes in serum immunoglobulin levels. It is hypothesized that the expression of the PR4 idiotype is due to certain germline genes preferentially expressed rather than being the result of polyclonal B cell activation.—Authors' Summary

Microbiology

Bhatia, V. N. and Nirmaladevi, B. Significance of neither green nor red (NGR) bacilli in FDA-EB staining. Indian J. Lepr. **63** (1991) 218–222.

This study deals with determination of viability by the FDA-EB method. It has been observed that some of the bacilli do not take any color in FDA-EB preparations. These can be called "neither green nor red" (NGR) bacilli. These nonstaining bacilli should be taken into account when reporting viability by FDA-EB method.—Authors' Abstract

Eiglmeier, K., Honore, N. and Cole, S. T. Towards the integration of foreign DNA into the chromosome of *Mycobacterium leprae.* Res. Microbiol. **142** (1991) 617–622.

Integrative plasmid vectors based on the pSAM2 system of *Streptomyces ambofaciens* offer great potential for the genetic analysis of *Mycobacterium leprae.* To assess this, the chromosomal attachment site of *M. leprae, att*-pSAM2, has been cloned, mapped and characterized. Nucleotide sequence analysis shows *att*-pSAM2 to correspond to a putative $tRNA^{pro}$ gene identical in sequence to those of *S. ambofaciens* and *M. tuberculosis.* In addition, it is shown that the genes encoding aspartate semialdehyde dehydrogenase, *asd,* and an anonymous protein antigen recognized by sera from leprosy patients, are linked to the *M. leprae att*-pSAM2 locus.—Authors' Summary

England, P. M., Wall, S. and McFadden, J. IS*900*-promoted stable integration of a foreign gene into mycobacteria. Mol. Microbiol. **5** (1991) 2047–2052.

An artificial mycobacterial transposon was constructed by placing two copies of the insertion sequence IS*900* flanking a kanamycin resistance gene into a non-(mycobacterial) replicating vector. Constructs were introduced into mycobacteria by electroporation and transposition events conferring kanamycin resistance were selected. Integration of IS*900* into several genomic sites was analyzed by Southern blotting and shown to involve both simple insertions and cointegrate formation, suggesting that IS*900* can transpose by a replicative mechanism. Kanamycin resistance of IS*900*-integrated transformants was shown to be stable in the absence of selection.—Authors' Summary

Espiritu, C. G., Gelber, R. and Ostler, H. B. Chronic anterior uveitis in leprosy: insidious cause of blindness. Br. J. Ophthalmol. **75** (1991) 273–275.

Chronic low-grade anterior uveitis is the commonest cause of blindness in leprosy. It is usually asymptomatic until the late

stages, and often patients seek help only after irreversible visual impairment has occurred. We present herewith several cases of this entity to emphasize the insidious nature of the disease, the extent of ocular damage it can cause, and the importance of early detection and treatment.—Authors' Abstract

Jayapal, V., Sharmila, K. M., Selvibai, G., Thyagarajan, S. P., Shanmugasundaram, N. and Subramanian, S. Fluorescein diacetate and ethidium bromide staining to determine the viability of *Mycobacterium smegmatis* and *Escherichia coli.* Lepr. Rev. **62** (1991) 310–314.

The ability of the fluorescein diacetate and ethidium bromide fluorescent staining method to assess the percentage of viable bacterial cells in suspension was compared with the plate counting method. *Mycobacterium smegmatis* and *Escherichia coli* bacterial cell suspensions were incubated at 60°C. At different time intervals samples were taken and the percentage of viable cells in each sample was assessed by the fluorescent staining method and compared with the plate counting method. The fluorescent staining method showed a positive correlation with the plate counting method. However, the viable counts by the plate counting method were lower than the staining method when incubated at 60°C, indicating a lag period in the decay of enzymes after bacterial death. Hence, the fluorescent staining technique can be used to assess the trend of bacterial death rather than to assess the exact number of viable bacilli.—Authors' Summary

McNeil, M., Daffé, M. and Brennan, P. J. Location of the mycolyl ester substituents in the cell walls of mycobacteria. J. Biol. Chem. **266** (1991) 13217–13223.

The question of the precise location of mycolic acids, the single most distinctive cell-wall entity of members of the *Mycobacterium* genus, has now been addressed. The free hydroxyl functions of the arabinogalactan component of the mycobacterial cell wall were *O*-methylated under conditions in which the mycolyl esters were not cleaved. Subsequent replacement of the mycolyl functions with *O*-ethyl groups resulted in an acid- and base-stable differentially *O*-alkylated surrogate polysaccharide, more amenable to analysis. Complete hydrolysis, reduction, acetylation, and gas chromatography/mass spectrometry revealed the unexpected finding that the mycolyl substituents were selectively and equally distributed on the 5-hydroxyl functions of terminal- and 2-linked arabinofuranosyl (Ara*f*) residues. Further analysis of the *O*-alkylated cell wall through partial acid hydrolysis, $NaB[^2H]_4$ reduction, pentadeuterioethylation, and gas chromatography/mass spectrometry demonstrated that the mycolyl units are clustered in groups of four on the previously recognized nonreducing terminal pentaarabinosyl unit [β-Ara*f*-(1 → 2)-α-Ara*f*)$_2$-3,5-α-Ara*f*. However, only about two thirds of the available pentasaccharide units are so substituted. Thus, the antigenicity of the arabinan component of mycobacterial cell walls may be explained by the fact that about one third of the pentaarabinosyl units are not mycolyated and are available for interaction with the immune system. On the other hand, the extreme hydrophobicity and impenetrability of the mycobacterial cell may be explained by the same motif also acting as the fulcrum for massive esterified paraffin residues. New fundamental information on the structure of mycobacterial cell walls will aid in our comprehension of its impenetrability to antibiotics and role in immunopathogenesis and persistence of disease.—Authors' Abstract

Orpiszewski, J., Hebda, C., Szykula, J., Powls, R., Clasper, S. and Rees, H. H. Multiple forms of *O*-methyltransferase involved in the microbial conversion of abietic acid into methyl abeitate by *Mycobacterium* sp. FEMS Microbiol. Lett. **82** (1991) 233–236.

Six out of seven tested strains of mycobacteria transformed abietic acid to methyl abietate in shake culture. The conversion carried out by *Mycobacterium* sp. MB 3683 was induced by the substrate and stimulated by methionine. Fractionation of the cell extract of *Mycobacterium* sp. MB 3683 on DEAE cellulose, Ultrogel AcA 44 and MONO Q resulted in the separation of three

distinct methyltransferase activities which could also esterify palmitic acid. The separated forms of the methyltransferase exhibited different activities toward these two substrates.—Authors' Summary

Palomino, J. C., Falconi, E., Marin, D. and Guerra, H. Assessing the viability of *Mycobacterium leprae* by the fluorescein diacetate/ethidium bromide staining technique. Indian J. Lepr. **63** (1991) 203–208.

In the present study we have evaluated the fluorescein diacetate/ethidium bromide (FDA/EB) staining technique to assess the viability of *Mycobacterium leprae* obtained from biopsies of leprosy patients under different periods of treatment. Bacillary suspensions were obtained from skin punch biopsies and stained with the FDA/EB solution. The average percentage of green cells seen which were deemed to be viable were: 67.2% of green cells in patients without previous treatment; 45.6% in patients with 1 to 6 months of treatment; 25.9% for patients with 7 to 12 months of treatment and 10.5% in patients with 13 to 24 months of treatment. All the patients studied were on multidrug therapy. The differences obtained in the percentages of green cells in the different groups of patients were statistically significant as determined by the Wilcoxon's test. The decrease in the percentage of green cells observed with increasing periods of treatment suggests that the FDA/EB technique correlates with the actual viability of *M. leprae.* The application of this technique in the routine procedures performed with Hansen's disease patients could be very useful for monitoring the effectiveness of treatment in leprosy patients.–Authors' Abstract

Patel, B. K. R., Banerjee, D. K. and Butcher, P. D. Extraction and characterization of mRNA from mycobacteria: implication for virulence gene identification. J. Microbiol. Methods **13** (1991) 99–111.

A method for the extraction of intact total RNA from mycobacteria (*Mycobacterium bovis* BCG and *M. leprae*) has been developed. The presence of specific mRNA transcripts within this population has been confirmed by the following evidence: hybridization of Northern blots with gene-specific probes revealed discrete size classes of transcript; increases in mRNA levels for the 71-kDa heat-shock protein were seen after heat shock and this was abolished with rifampin; cDNA specific for hsp71-kDa mRNA could be synthesized from total RNA and characterized after PCR amplification. The ability to analyze mRNA by this method has implications for the study of gene expression and the molecular mechanisms of pathogenicity in mycobacteria.—AS (Trop. Dis. Bull.)

Wheeler, P. R. and Ratledge, C. Phospholipase activity of *Mycobacterium leprae* harvested from experimentally infected armadillo tissue. Infect. Immun. **59** (1991) 2781–2789.

Three types of phospholipase activity—phospholipase A_1, A_2, and lysophospholipase—were detected in *Mycobacterium leprae* harvested from armadillo tissue at about 25% of the specific activity found in a slowly growing mycobacterium, *Mycobacterium microti,* which was grown in medium to optimize its phospholipase activity. The highest activity found was lysophospholipase, which released fatty acid from 2-*lyso*-phosphatidylcholine. Phospholipase activity was detected by using phosphatidylcholine and phosphatidylethanolamine. Differences in relative activities with these three types of substrate distinguished phospholipase activity in *M. leprae* extracts from armadillo liver extracts. Furthermore, retention of activity in *M. leprae* after NaOH treatment showed that the activity associated with *M. leprae* was not host derived. The specific activity of phospholipase was 20 times higher in extracts of *M. leprae* than in intact *M. leprae* organisms. Diazotization, a treatment which abolishes activities of surface enzymes exposed to the environment by the formation of covalent azide bonds with exposed amino groups, did not affect *M. leprae*'s phospholipase activity, with one exception: release of arachidonic acid from phosphatidylcholine, which was partially inhibited. Phenolic glycolipid-I, the major excreted amphipathic lipid of *M. leprae,* inhibited phospholipase activity, including release of arachidonic acid, for both *M. lep-*

rae- and armadillo-derived activity.—Authors' Abstract

Young, D. B. and Garbe, T. R. Heat shock proteins and antigens of *Mycobacterium tuberculosis.* Infect. Immun. **59** (1991) 3086–3093.

The heat-shock response of *Mycobacterium tuberculosis* has been characterized in detail by one- and two-dimensional polyacrylamide gel electrophoresis after metabolic labeling with [^{35}S]methionine and ^{14}C-amino acids. A temperature increase from 37°C to 42°C induced elevated synthesis of three major proteins corresponding to the DnaK, GroEL, and GroES proteins of *M. tuberculosis* previously identified as prominent antigens. At higher temperatures (45°C to 48°C), synthesis of GroEL decreased and novel heat-shock proteins with molecular masses of 90, 28, 20, and 15 kDa were observed. These new proteins did not comigrate with known antigens during two-dimensional gel electrophoresis. The heat-shock response is discussed with regard to the possible importance of transcriptional regulation of mycobacterial genes *in vivo.*—Authors' Abstract

Experimental Infections

Job, C. K., Drain, V., Williams, D. L., Gillis, T. P., Truman, R. W., Sanchez, R. M., Deming, A. T. and Hastings, R. C. Comparison of polymerase chain reaction technique with other methods for detection of *Mycobacterium leprae* in tissues of wild nine-banded armadillos. Lepr. Rev. **62** (1991) 362–373.

Thirty, nine-banded armadillos weighing between 3 and 5 kilograms trapped from an area endemic for armadillo leprosy were collected at random; killed, autopsied and examined histopathologically. Also, one of the right inguinal lymph nodes was removed under sterile precautions and examined using polymerase chain reaction (PCR), direct smear examination, mouse foot pad study, culture in laboratory media, and histopathology with a view to detecting *Mycobacterium leprae.* Blood was collected at death and tested for IgM antibodies to PGL-1.

According to the PCR study of the inguinal lymph nodes, 16 of 30 armadillos (53.3%) had evidence of *M. leprae.* Significant levels of IgM antibodies to PGL-1 and identifiable lepromatous granuloma in inguinal lymph nodes were found in 2 animals (6.7%) with advanced disseminated disease. The prevalence of generalized leprosy according to autopsy study was 13.3%; according to histopathological examination of ear tissue, 3.3%. The presence of *M. leprae* in the tissues evoked no special tissue reaction in the early stages. The pattern of spread of the disease in 2 animals closely resembled that found in experimental animals infected intracutaneously. Initiation of infection by inoculation of *M. leprae* through thorn pricks remains a distinct possibility.—Authors' Summary

Job, C. K., Sanchez, R. M. and Hastings, R. C. An attempt to produce experimental tuberculoid leprosy in the nine-banded armadillo. Indian J. Lepr. **63** (1991) 159–165.

In an attempt to produce experimental tuberculoid leprosy, 3 nine-banded armadillos, 2 with borderline tuberculoid lepromin reaction, and 1 with tuberculoid lepromin reaction, were chosen. They were injected subcutaneously in a 4 sq. cm area in the abdominal skin with saline suspension of 6.5×10^7 *Mycobacterium leprae.* Induration of skin at the injected site appeared in 24 hr and persisted for 6 mo in one and for 18 mo in the other two animals. Histopathological examination of the infected site at 6 weeks, 18 and 20 mo showed progressively decreasing granulomatous inflammation; but the cutaneous nerves were uninvolved. Autopsy examination of the three animals failed to show disseminated disease. Since there was no evidence of nerve involvement, experimental transmission of

tuberculoid leprosy to armadillos could not be established in this study.—Authors' Abstract

Kohli, M., Sharma, V. K., Vaishnavi, C., Ganguly, N. K., Kaur, S. and Chugh, S. Renal brushborder membrane vesicle study of marker enzymes and uptake of nutrients in *Mycobacterium leprae* infected mice. Jpn. J. Exp. Med. **60** (1990) 285–290.

The renal brush-border membrane vesicles (BBMV) were used to elucidate the early biochemical functional status during the course of experimental *Mycobacterium leprae* infection in mice. The activities of the characteristic brush-border enzymes viz: alkaline phosphatase, leucine amino peptidase and γ-glutamyl transpeptidase were found to be significantly decreased ($p < 0.001$) at 3 and 6 months after infection. The transport of nutrients viz: D-glucose, L-alanine, L-lysine and L-aspartate across BBMV showed a similar pattern. The activity of brush-border enzymes and transport of nutrients across the membrane returned to normal at 9 months postinfection, suggesting regeneration of the brush-border membrane.—Authors' Summary

Singh, I. G., Mukherjee, R. and Talwar, G. P. Resistance to intravenous inoculation of *Mycobacterium tuberculosis* H37Rv in mice of different inbred strains following immunization with a leprosy vaccine based on *Mycobacterium w*. Vaccine **9** (1991) 10–14.

Four strains of mice, namely, BALB/c, C57BL/6 NCrl (*Bcg*^{s}), C3H/He NCrl and CBA/N (*Bcg*^{r}) were experimentally infected with *Mycobacterium tuberculosis* H37Rv to induce sublethal infection. The level of infection was assessed by screening tuberculin reaction, pulmonary lesions, and viable units of mycobacteria recovered from the lung, spleen and liver. On prior immunization with 10^7 heat-killed suspension of *Mycobacterium w*, an antileprosy vaccine currently under large-scale human trials in India, protection was observed against tuberculosis in all four strains of mice used in the study as assessed by significant reduction of both pulmonary lesions and viable units of mycobacteria recovered from different organs. In parallel experiments, live BCG was able to confer protection to mice of Bcg^s strains but not to mice of the Bcg^r strains. Results of these experiments suggest that a vaccine based on heat-killed *Mycobacterium w* has the potential also to confer protection against tuberculosis in mice of genetic strains whose immune system is less triggered by intravenous injection of viable BCG.—Authors' Abstract

Tomioka, H., Sato, K. and Saito, H. Comparative *in vitro* and *in vivo* activity of fleroxacin and ofloxacin against various mycobacteria. Tubercle **72** (1991) 176–180.

In vitro antimicrobial activity of fleroxacin (6, 8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid) and ofloxacin against representative pathogenic mycobacteria was evaluated by the agar dilution method, using 7H11 agar medium. Fleroxacin showed appreciable antimicrobial activity against *Mycobacterium tuberculosis* (MIC_{90} = 6.25 mg/l), *M. kansasii* (MIC_{90} = 3.13 mg/l), and *M. fortuitum* (MIC_{90} = 6.25 mg/l); whereas *M. marinum, M. scrofulaceum, M. avium, M. intracellulare,* and *M. chelonei* were highly resistant to the agent. The activity of fleroxacin was comparable to that of ofloxacin. Fleroxacin showed antimicrobial activity against *M. intracellulare* phagocytosed in murine peritoneal macrophages at a concentration of 10 mg/l in the culture medium, but its activity was considerably lower than that of ofloxacin. On the other hand, the therapeutic activity of fleroxacin against *M. fortuitum* infection induced in mice was higher than that of ofloxacin. Neither fleroxacin nor ofloxacin was efficacious against *M. intracellulare* infection. Fleroxacin significantly depressed the growth of *M. leprae* in the mouse foot pad.—Authors' Summary

Epidemiology and Prevention

Almodovar, P. I. and Figueroa, J. Leprosy in Puerto Rico: a decade later. Bol. Asoc. Med. P.R. **82** (1990) 466–468.

This paper describes progress in the control of leprosy in Puerto Rico in the period 1981–1989, during which 75 new cases were diagnosed. Data were obtained from the Tropical Diseases Clinic in the University of Puerto Rico School of Medicine and from the National Hansen's Disease Program Registry at Carville, Louisiana, U.S.A. The very considerable contribution of the latter center in attempting to localize all patients of Puerto Rican origin is acknowledged by the authors. The data, which are presented in some detail, indicate a decrease of both incidence and prevalence of leprosy. Patients are getting older and tend to die from natural causes and the number of new cases diagnosed yearly is now less than half that seen in the previous decade. The improvement is attributed to the effectiveness of treatment and better socioeconomic conditions.—A. C. McDougall (Trop. Dis. Bull.)

Crook, N., Ramasubban, R., Samy, A. and Singh, B. An educational approach to leprosy control: an evaluation of knowledge, attitudes and practice in two poor localities in Bombay, India. Lepr. Rev. **62** (1991) 395–401.

Based on the hypothesis that a systematic, carefully planned, educational approach to leprosy would yield results in terms of knowledge, attitudes, and case presentation superior to those of the established and traditional mass survey method, ALERT-India launched a program in S ward of Bombay in February 1985 to compare the two. An intensive program of health education, using trained teams, was carried out in one zone of this ward over a period of 12 months. Eight months later, mass survey work (as used routinely in previous years and on a country-wide basis) was carried out in an adjacent zone. In 1987, the Centre for Social and Technological Change in Bombay, in association with the School of Oriental and African Studies, University of London, was requested to evaluate the effect of the above educational approach in terms of knowledge, attitudes, and practice in both the trial and control zones. Other aspects of this experimental approach, including its cost and effectiveness in identifying cases of leprosy, will be published separately. The design of the "KAP" evaluation and the social and environmental controls introduced in the statistical analysis are described. The results pointed to a considerable degree of ignorance about leprosy as a disease (and its treatment) in both the study and the control zones. Knowledge about early symptoms was particularly weak, and on all aspects scores for women were invariably lower than men. General education enhanced the absorption of specific knowledge, and the education of children compensated adequately for lack of parental education in this respect. Overall the evaluation indicated that the intensive educational approach was superior to the survey approach in terms of improving knowledge, attitudes, and practice.—Authors' Summary

Kumar, A., Durgambal, K., Kalaivani, S. and Sirumban, P. The factors influencing the operational efficiency of leprosy case detection programme. Indian J. Lepr. **63** (1991) 180–194.

Under our National Leprosy Eradication Programme (NLEP), leprosy cases are being detected by paramedical workers by conducting population surveys. In order to detect the leprosy cases early, for their timely antileprosy treatment, it is necessary that the leprosy surveys are implemented and supervised efficiently. However, present experience indicates that the existing survey efficiency needs to be improved, for which it is necessary to analyze the factors which may interfere with the optimal survey efficiency of paramedical workers. An attempt has been made through present piece of work to identify such factors in relation to a) the paramedical workers and survey facilities, b) the implementation and supervision of leprosy survey, and c) the community involved in survey. These factors are discussed in detail to assist the NLEP admin-

istrators in devising a suitable action plan to improve leprosy case detection efficiency.—Authors' Abstract

Louis, J. P., Merlin, M., Josse, R., Trebucq, A., Drevet, D., Desfontaine, M., Hamono, B., Coddy-Zitsamele, R., Sima, A., Hengy, C., Gelas, H. and Cottenot, F. [Surveys of prevalence of leprosy in 5 countries of Central Africa.] Acta Leprol. (Genève) **7** (1991) 347–350. (in French)

The authors report the results of national surveys conducted in five Central Africa states: Cameroon, Congo, Gabon, Equatorial Guinea, and RCA. The method used was cluster sampling among random populations. Only adults (> 15 years of age) took part in the study. The prevalence rates were between 6 and 14 per thousand. They are markedly higher than the official data.—Authors' English Summary

Nair, N. G. K., Radhakrishna, S., Ramakrishnan, R. and Sreenivas, V. Some indices pertaining to the leprosy control programme in Tamil Nadu, based on data from the random sample of fourteen government control units. Indian J. Med. Res. [A] **93** (1991) 208–216.

From a random sample of 14 government leprosy control units in Tamil Nadu, information on the profile of the newly diagnosed leprosy patients and some important aspects of the control program in 1978–1981 was collected when monotherapy with dapsone was the practice. Among the new patients, 55% were males, 24% were children, 6% had lepromatous leprosy and 9% had a deformity. About 65% were detected by active case-finding methods and 25% were voluntary referrals. Of the total diagnosed patients, only 68% started treatment; further, of these, about 40% collected drugs for at least 6 months in the first year of treatment. The average attendance at the clinic was 34% of the due attendance. Coverage in the annual examination of family contacts was 57%. During the 4 yr period, about 70% of the villages had population surveys with a coverage of 75% or more. The introduction of multidrug therapy has provided a new impetus to the program and therefore a similar study is called for to provide valuable information about the extent of improvement in completion rates and overall impact.—Authors' Abstract

Opromolla, D. V., Nobrega, R. C., da Silva e Gonçalves, N. N., Padovani, S. H. P., Padovani, C. R. and Gonçalves, A. [An estimate of the prevalence of hanseniasis by means of investigation into nonspecific demand for health services.] Rev. Saúde Pública **24** (1990) 178–185. (in Portuguese)

In view of the importance of knowing the prevalence and incidence rates of a disease to learn about its behavior and control at the collective level, a study was undertaken to determine the occurrence of hanseniasis among the clients of health agencies and to explore the use of this methodology for estimating the epidemiologic "iceberg" of the disease, i.e., the total number of cases including those that are not officially reported. The city of Taubaté in the Paraíba Valley, State of Sao Paulo, Brazil, was chosen for the study. All clients aged 15 years or older were screened regardless of variables such as sex, age, social condition or marital status. The study was based on what is known about the populational distribution of the disease and the characteristics of health services. In view of the local peculiarities of operationalization (e.g., identity of the different clientelles, availability of offices, hours of greatest flux), the sampling process used was simple randomization. The patients with active disease detected, 40 of the 10,013 persons examined, correspond to a prevalence of 3.99/1000, with a confidence interval (at the 5% level of reliability) of 3365 to 4625/1000, indicating that the minimum estimated increase of prevalence is of the order of 52% and the maximum estimated increase is of the order of 109%. The indeterminate form of the disease predominated among the patients (35.00%), and this predominance was even more explicit when the patients were classified as registered or new cases: in this latter category, the indeterminate form reached 56.53% and its distribution by age range was close to that observed in the officially recorded data, as verified by comparing observed cases with expected cases calculated from population

indicators. As to sex ratio, a predominance of males was observed.—Authors' English Abstract

Pattyn, S. R. Evolution of the leprosy problem in some African areas during the 1980–89 decade. (Editorial) Ann. Soc. Belg. Med. Trop. **71** (1991) 1–4.

Professor Pattyn describes the evolution of the leprosy problem since 1980 in intensive programs of diagnosis and treatment in République Fédérale Islamique des Comores, Burundi, Rwanda and two regions of Zaïre. By 1982–1983 it became clear in all these areas that some decisive step had to be taken for the management of large numbers of "old cases" in patients who had taken dapsone for years and who had no clinical activity, with negative bacteriological findings. Except in Rwanda, it was decided to give all these patients a single dose of 1500 mg rifampin and to declare them bacteriologically cured, but invite them to report back if they suspected relapse. In Burundi, the relapse rate was 1.2/100 patient-years of follow-up; in the Haut Zaïre region it was 1.4/100 patient-years of follow-up; in Rwanda, the Ministry of Health recently decided to stop all treatment for bacteriologically negative old multibacillary patients who had received dapsone for 10 years or more, and the outcome has yet to be assessed. On Grande Comore (Comores), only 1 case has been diagnosed between 1982 and 1989 and it has been concluded that the island is leprosy-free. The author discusses the possible influence of chemotherapy in these regions and goes on to draw attention to (1) the lack of any measurable reduction during the decade in the detection rate, and (2) the high infection rate in children in Anjouan (Comores). The editorial includes an interesting discussion of the possible implications of the theory that adult leprosy is mostly the result of childhood infection.—From Trop. Dis. Bull.

Ree, G. H. Pattern of leprosy in Queensland, Australia, 1855–1990. Lepr. Rev. **62** (1991) 420–430.

Leprosy was first diagnosed in Queensland in 1855. From then until 1990, 929 patients with the disease were notified. The pattern of notification has varied with the passage of time, and with the changing pattern of migration into Queensland. In the early days, Chinese, Melanesians and Caucasians featured prominently. The first Aboriginal notification was in 1892. In the latter part of this century, significant numbers of Torres Strait Islanders and migrants from South East Asia have been recorded. Among Caucasians, the incidence peaked in the decade 1931–1940, although the prevalence rate in this population remains much higher than in Causcasians. The control of leprosy is at a high level in Queensland today, but there is a continuing low level of new case reporting, many of them imported.—Author's Summary

Samy, A. A., Mancheril, J., Manek, K. P. and McDougall, A. C. ALERT-India 1981–89: nine years' experience in leprosy control in the slums of Bombay. Lepr. Rev. **62** (1991) 315–328.

Bombay has a population of about 8 million people, one half of whom live in slums. In 1981, ALERT-India started its first leprosy control project in N, S and T Wards of Greater Bombay Municipal Corporation covering an area of 122 sq km in the north-eastern suburbs of Vidhyavikar, Ghatkopar, Vikhroli, Kanjurmarg, Bhandup and Mulund, with a total population of 1,100,000 according to the 1981 census. In the 9 years of operation, over 12,000 patients have been registered and treated, and of these 7425 have been released from treatment, having satisfactorily completed courses of chemotherapy. However, over 1000 cases are still identified every year by house-to-house or school surveys, or by self-reporting, including a considerable percentage in children. The origin, development, staff structure, operational procedure, administration and recording system of ALERT-India are described in detail, with emphasis on what has been accomplished with purely outpatient facilities, using paramedical workers, all of whom have received inservice training from government-recognized training centers for their specific tasks. The account includes a brief description of an expansion of the organization's work into townships in New Bombay, where prelim-

inary surveys in 1988 confirmed the presence of leprosy cases and the need for treatment facilities. The discussion addresses: 1) the better use of the large volume of statistical information which has been collected by ALERT-India during the past 9 years, with emphasis on its value in assessing the impact on the control program and modifying future policy; 2) the need to radically examine the present policy of survey, versus an "education campaign approach" with regard to increasing early case-detection and self-reporting; 3) the establishment of a central coordinating body for leprosy control in Bombay to exchange information, coordinate efforts and formulate a future plan of action, the latter in association with the National Leprosy Eradication Programme; and 4) the development of a health education resource center in association with the Bombay Municipal Corporation.—Authors' Summary

Sun, H.-T., *et al.* [Analysis of 335 new outpatient cases of leprosy.] China Lepr. J. 7 (1991) 79–81. (in Chinese)

In 1979–1988, the outpatient department for leprosy of Sichuan Provincial Institute of Skin Diseases found 335 new cases of leprosy, of which male versus female is equal to 7.8:1, 87.5% were 15–44 years of age, 58.8% were from leprosy hypo- and nonendemic areas, 63% were early cases, 68.7% had four swollen nerves or more, 68.7% were lepromatous, 57.6% and 73.8% had lesions on their faces and extremities, respectively. The majority of them had been misdiagnosed as other diseases over several years in general hospitals including provincial hospitals, and each of them had been misdiagnosed on average of 4.3 times. The authors think that in leprosy hypo- and nonendemic areas of Sichuan the ability to find cases of leprosy is low, and it is necessary to make great efforts for popularizing knowledge of leprosy to medical workers at various levels so as to increase their ability to make the right diagnosis.—Authors' English Abstract

Truman, R. W., Kumaresan, J. A., McDonough, C. M., Job, C. K. and Hastings, R. C. Seasonal and spatial trends in the detectability of leprosy in wild armadillos. Epidemiol. Infect. **106** (1991) 549–560.

A survey for leprosy among 565 armadillos from Louisiana and Texas found IgM antibodies to the phenolic glycolipid-I antigen of *Mycobacterium leprae* in 16% of the animals. There were no geographic trends in the distribution of prevalence rates between the sites, and the disease probably has a much greater range. Repeat observations in one location showed significant seasonal variations in the observable antibody prevalence rate, but the yearly average remained similar. Infected armadillos tended to be heavier, and the females usually had plasma progesterone concentrations indicative of sexual maturity. Using these characteristics to stratify the populations into adult and subadult cohorts, variations in the observable leprosy prevalence rate were seen to be proportional to changes in the age structure of the populations. Leprosy appears to be maintained in steady state within some regions, and nearly a third of the adult armadillos in Louisiana and Texas harbor *M. leprae.*—Authors' Summary

Rehabilitation

Carayon, A. and Chevallard, A. [Progressive dislocations of the foot joints in leprosy.] Rev. Chir. Orthop. **76** (1990) 579–582. (in French)

The authors have observed 135 dislocations of the tarsus in leprosic feet. They recall the mechanism of the lesion and propose a classification. Forty-two cases were operated on to prevent further deformities. When early treated limited arthrodeses may be beneficial in some cases. In more severe cases rebuilding arthrodesis alone or associated to cancellous bone grafting is indicated. In very severe cases amputation is only indicated because the Wladimiroff-

Mickulicz technique as well as grafts lead to failures related to the metatarso-phalangeal joints stiffness.—Authors' English Summary

Naik, S. S., Hambarde, P. S. and Desai, A. N. Problems and needs of women leprosy patients in Bombay and Goa—a preliminary report. Indian J. Lepr. **63** (1991) 213–217.

By studying the status of 151 women leprosy patients (24 from a leprosy asylum and 127 attending urban leprosy centers at Goa and Bombay), it was noticed that a sizeable proportion experienced problems in society ascribable to the disease, especially at the initial stages of the disease. However, most of them seemed to have managed to settle well in their families as housewives subsequently. Younger women leprosy patients expressed the need for financial assistance for completing their own education and for starting small-scale business. The older women were more interested in educating their children.—Authors' Abstract

Ramanathan, U., Malaviya, G. N., Jain, N. and Husain, S. Psychosocial aspects of deformed leprosy patients undergoing surgical correction. Lepr. Rev. **62** (1991) 402–409.

A psychosocial study was conducted on 25 randomly selected leprosy patients undergoing corrective surgical procedures for their deformities. High anxiety and depression levels found preoperatively were reduced significantly after operation. Psychiatric assistance is needed for these patients in order to clear their psychic aberrations, create awareness, boost morale, and to give self-confidence. Only 50%–75% of preoperative expectations were satisfied, but that was only in 40% of patients. This calls for a preoperative counselling session with the patients to help them reach the realistic goals that they can achieve. They should be told what benefits surgery can offer them and be made aware of the problems which will persist after operation, such as anesthesia and analgesia.—Authors' Summary

Yan, L.-B., *et al.* [Clinical application of Modulan in leprosy.] China Lepr. J. **7** (1991) 77–78. (in Chinese)

Modulan is a kind of plastic that can be used for moulding the handles of tools according to the palm shape of a user. The authors present the results of using Modulan for 53 patients with leprosy, covering 649 pieces of 15 kinds of tools for their life and labor. The users think that it can strengthen the functions of the hands in all users and it has the effect of heat insulation and protection against scalding in 80%. It is simple, convenient and durable to use in 74%; 95% of the users said that they are pleased with its use.—Authors' English Abstract

Zhang, G.-C., *et al.* [Epidemiological investigation of disability in leprosy. (II) Analysis of disability.] China Lepr. J. **7** (1991) 67–72. (in Chinese)

In a survey of 14,257 leprosy patients in Yangzhou City (10 counties) and Dongtai City of Jiangsu Province, 8122 disabled cases have been found, of which 62.04% have deformity of WHO degree III. Only a minority of eyes, hands, and feet with the deformities are suited to orthopedy. The authors think that to prevent deformity the most important thing is to popularize knowledge of leprosy.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Alexandrova, A. E., Shcherbakova, N. M., Zabolothykh, N. V. and Shchegoleva, R. A. [Use of dimephosphone in a pathogenetic therapy of experimental tuberculosis.] Probl. Tuberk. **8** (1991) 68–71. (in Russian)

The results of a study of a new synthetic drug, dimephosphone, used as a pathogenetic means in the treatment of experimental tuberculosis are presented. Dimephosphone was found to be responsible for both the *in vivo* and *in vitro* decrease of the degree

of MBT resistance to rifampin. The findings of macroscopic, histologic and bacteriologic examinations demonstrated a significant increase in the effectiveness of antituberculous therapy. Dimephosphone monotherapy in mice elicited manifested stimulation of peritoneal macrophages: increase in O_2^- production, and decline in extracellular 5-nucleotidase activity. Nemolysine-synthetic cellular splenic activity in mice rose essentially. No direct stimulating influence of dimephosphone on functional macrophage activity *in vitro* was found.—Authors' English Abstract

Buseva, T. A. and Koryakin, V. A. [A thermoindicative nasal test for detecting allergy to antituberculosis drugs.] Probl. Tuberk. **6** (1991) 35–37. (in Russian)

A diagnostic test has been suggested for the detection of the allergic-type adverse reaction to antituberculosis drugs. The drug-allergen is applied to the nasal mucosa and the temperature reaction of the alla nasi tissues is measured with the help of a mesomorphic thermoindicative film.—Authors' English Abstract

Castro, A. G., Esaguy, N., Macedo, P. M., Aguas, A. P. and Silva, M. T. Live but not heat-killed mycobacteria cause rapid chemotaxis of large numbers of eosinophils in vivo and are ingested by the attracted granulocytes. Infect. Immun. **59** (1991) 3009–3014.

We studied leukocyte chemotaxis triggered by a local injection of mycobacteria (*Mycobacterium avium* and *M. smegmatis*) in BALB/c and C57BL/6 mice. Our experimental model consisted of the induction of a subcutaneous air pouch in the dorsal area of mice and inoculation 6 days later of 10^8 CFU of mycobacteria. Inflammatory exudates were harvested from the air pouch cavities 15, 30, and 45 min after the injection of the inocula. Injection of the microorganisms resulted in the migration of an elevated number of eosinophilic granulocytes into the inflammatory cavities. At 30 min after the inoculation of the mycobacteria, the air pouches contained between $(3.9 \pm 0.3) \times 10^5$ (*M. avium*) and $(3.3 \pm 0.3) \times 10^5$ (*M. smegmatis*) eosinophils, corresponding to more than one third (41.4% to 38.3%) of the leukocytes present in the inflammatory cavities. Less than one half of the eosinophils were attracted to the air pouches when the same number of heat-killed mycobacteria were inoculated [$(1.3 \pm 0.2) \times 10^5$ cells for *M. avium* and $(1.5 \pm 0.2) \times 10^5$ cells for *M. smegmatis*]. Injection of gram-negative bacteria (*Escherichia coli*), of latex beads, or of casein resulted in the attraction of inflammatory eosinophils in numbers that were comparable to those attracted by the heat-killed mycobacteria. Our data document the fact that live mycobacteria exert a rapid chemotactic effect on eosinophils. We therefore postulate that mycobacteria either contain or induce the production of an eosinophilotactic factor. Because this chemotactic effect occurs during the acute inflammatory response to mycobacteria, it cannot be due to the formation of immune complexes (a major infection-associated chemotactic factor for eosinophils). The attracted eosinophils had an important role in the local phagocytosis of mycobacteria, as indicated by our finding, derived from thin-section electron microscopy quantifications, that at 30 min after *M. avium* inoculation the inflammatory exudates contained $(2.2 \pm 0.5) \times 10^5$ mycobacterium-bearing eosinophils (corresponding to 57% of the total eosinophils), as compared with $(2.1 \pm 0.1) \times 10^5$ neutrophils and $(1.5 \pm 0.2) \times 10^5$ macrophages with ingested bacilli. We conclude that mycobacteria induce the attraction of eosinophils to inflammatory sites and that these granulocytes have the capacity to phagocytize these bacilli *in situ*.—Authors' Abstract

Chandrasekhar, S. and Perumal, V. K. Association of increased mycobacterium growth inhibitory factor with antituberculous immunity. Eur. Respir. J. **4** (1991) 783–788.

Cell-mediated-immune mechanisms (CMI) were studied in 51 patients with pulmonary tuberculosis to evaluate the role of mycobacterium growth inhibitory factor in prognosis of the infection, before and after the administration of antitubercular drugs. Twenty-five Mantoux-negative individuals who were subsequently bacille Calmette-

Guérin (BCG) vaccinated and 25 Mantoux-positive, nontuberculous controls were included in the study. Their clinical assessment was compared with skin sensitivity (Mantoux); lymphocyte transformation (LT) after stimulation with phytohemagglutinin (PHA) and purified protein derivative (PPD); macrophage migration inhibitory factor (MIF), mycobacteria growth inhibitory factor (Myco IF) and listerial growth inhibitory factor (List IF). The tests were carried out at the beginning of the treatment and at intervals of 3 months, extending to 1 year. In the case of Mantoux-positive controls, tests were carried out only once. It was found that Mantoux reaction had no correlation with LT, MIF, Myco IF and List IF. Both MIF and Myco IF were significantly elevated in improving patients; whereas increase in List IF was not significant. An important finding was that Myco IF was at a higher level in improving patients, whereas in those not responding to chemotherapy it was low.—Authors' Abstract

Content, J., De la Cuvellerie, A., De Wit, L., Vincent-Lévy-Frébault, V., Ooms, J. and De Bruyn, J. The genes coding for the antigen 85 complexes of *Mycobacterium bovis* BCG are members of a gene family: cloning, sequence determination, and genomic organization of the gene coding for antigen 85-C of *M. tuberculosis.* Infect. Immun. **59** (1991) 3205–3212.

A gene encoding the 33-kDa secreted protein of *Mycobacterium tuberculosis* (antigen 85-C) was isolated and sequenced. The corresponding DNA sequence contains a 1020-bp coding region. The deduced amino acid sequence corresponds to a 340-residue protein consisting of a 46-amino-acid signal peptide and a 294-amino-acid mature protein. Comparison with previously described genes for the 30-kDa antigen (the α antigen of *M. bovis* BCG, also called antigen 85-B) and the 32-kDa antigens from *M. bovis* BCG and *M. tuberculosis* (antigens 85-A) indicates that the three genes share considerable sequence homology (70.8% to 77.5%) but may also code for distinctive epitopes. Strong differences among the three sequences are clearly visible upstream and downstream from the region coding for the mature proteins. The three genes have been detected in the genome of *M. bovis* BCG by Southern blot hybridization with three type-specific probes. Furthermore, hybridization of large DNA fragments (100 to 1000 kbp) from *M. tuberculosis* separated by pulsed-field gel electrophoresis showed that the three genes coding for the antigen 85 complex are not clustered within the bacterial genome.—Authors' Abstract

Dautzenberg, B., Truffot, C., Legris, S., Meyohas, M.-C., Berlie, H. C., Mercat, A., Chevret, S. and Grosset, J. Activity of clarithromycin against *Mycobacterium avium* infection in patients with the acquired immune deficiency syndrome; a controlled clinical trial. Am. Rev. Respir. Dis. **144** (1991) 564–569.

Disseminated *Mycobacterium avium* infection is common in patients with acquired immune deficiency syndrome (AIDS), but no drug studies have been reported establishing antimicrobial activity against this organism in a controlled, randomized trial. Clarithromycin, a new macrolide, has activity against *M. avium in vitro* and in animals, but it has not been studied in humans. In this randomized, double-blind, placebo-controlled trial, we measured the ability of clarithromycin to reduce *M. avium* bacteremia in patients with AIDS and disseminated infection. Of 23 patients initially enrolled, 15 men with late-stage AIDS qualified for the study. One group received clarithromycin alone for 6 wk, then placebo plus rifampin, isoniazid, ethambutol, and clofazimine for 6 wk. The other group received placebo alone, then clarithromycin plus the other four drugs. Colony-forming units (CFU) of *M. avium* per milliliter of blood were determined by quantitative cultures taken at baseline and every 2 wk thereafter. Minimum inhibitory concentration of clarithromycin for 90% of the strains isolated from patients at baseline, as measured on 7H11 agar at pH 6.6, was 8 μg/ml. Eight eligible patients with initial positive cultures who were initially receiving clarithromycin alone had marked declines in blood *M. avium* CFU; in six cases, CFU decreased to zero. When seven patients were switched to placebo plus the other four drugs, CFU

rose in four patients and remained undetectable in three. The five eligible patients initially treated with placebo had progressive CFU increases; when three were switched to clarithromycin plus the four drugs, their CFU declined. We conclude that clarithromycin has consistent activity against *M. avium* and may benefit patients with AIDS and disseminated *M. avium* infection.—Authors' Summary

David, M., Lubinsky-Mink, S., Ben-Zvi, A., Suissa, M., Ulitzur, S. and Kuhn, J. Citrate synthase from *Mycobacterium smegmatis*; cloning, sequence determination and expression in *Escherichia coli*. Biochem. J. **278** (1991) 225–234.

A *Mycobacterium smegmatis Pst*I library was constructed by cloning these fragments downstream from the *lac* promoter of the expression vector pHG171. Three identically sized clones were isolated by complementation of an *Escherichia coli* strain (χ2338) deficient in citrate synthase. One insert (pBL265) was used in hybridization experiments with DNA from *E. coli* and *M. smegmatis* and it was demonstrated that the clones were indeed from *M. smegmatis*. The transcription of the *M. smegmatis* citrate synthase gene in *E. coli* relied upon the *lac* promoter. In translation experiments performed *in vitro* pBL265 gave rise to a novel protein of about 42 kDa. This band was not seen in "opposite-orientation" subclones. Various subclones in which the 5′-end was shortened nevertheless complement *E. coli* χ2338 and produce the 42 kDa protein. This demonstrates that the *M. smegmatis* citrate synthase gene uses its own ribosome-binding site in *E. coli*. The relevant 1.8 kb of the 2.8 kb insert was sequenced. A consensus *E. coli* ribosome-binding site was found centred precisely 10 bp upstream of the methionine codon. Other interesting features revealed by the sequence are discussed. Citrate synthase activity was assayed *in vitro* and the mycobacterial enzyme was found to be similar to those of the gram-positive bacteria.—Authors' Abstract

Del Portillo, P., Murillo, L. A. and Patarroyo, M. E. Amplification of a species-specific DNA fragment of *Mycobacterium tuberculosis* and its possible use in diagnosis. J. Clin. Microbiol. **29** (1991) 2163–2168.

In recent work, a species-specific *Mycobacterium tuberculosis* DNA fragment was cloned and sequenced. On the basis of its nucleotide sequence, two oligonucleotides were synthesized and used as primers for polymerase chain reaction (PCR) amplification. A 396-bp fragment was specifically amplified from the *M. tuberculosis* genome. No amplification was observed from any of 10 different mycobacterial strains, including those belonging to the *M. tuberculosis* complex. Neither was this fragment amplified from genomes of humans or different species of clinically important bacteria. The PCR product was detected by dot blot hybridization even when as little as 10 fg of purified *M. tuberculosis* DNA was used. This amplification method was subsequently used to detect and identify bacilli in different clinical samples, such as sputum, urine, and cerebrospinal fluid. A good correlation was observed between the results obtained with the PCR method that we describe and other diagnostic tests currently used. Thus, PCR amplification of this genomic fragment is proposed as a specific, rapid, and sensitive test for the diagnosis of infection with *M. tuberculosis*.—Authors' Abstract

Falla, J. C., Parra, C. A., Mendoza, M., Franco, L. C., Guzman, F., Forero, J., Orozco, O. and Patarroyo, M. E. Identification of B- and T-cell epitopes within the MTP40 protein of *Mycobacterium tuberculosis* and their correlation with the disease course. Infect. Immun. **59** (1991) 2265–2273.

Synthetic peptides derived from the amino acid sequence of MTP40, a recently characterized *Mycobacterium tuberculosis* protein, were tested by two different immunological assays in 91 individuals. For the purposes of this study, the population was distributed in four groups: active tuberculosis (TBC) patients with elevated bacillus loads (BK+), active TBC patients with low bacillus loads (BK−), healthy individuals living in the same household with tuberculous patients (HH), and normal individuals, who had presumably never been in contact with the bacilli (control). We found

that T cells of individuals belonging to the HH group showed the highest and most frequent recognition of these peptides in a T-cell proliferation assay, while their antibodies showed the lowest recognition of these peptides when tested by enzyme-linked immunosorbent assay. In contrast, TBC patients revealed an inverse pattern of immune response. Interestingly, one of these peptides (P7) was recognized by T cells of 64% of the HH individuals and by 4.5% of normal donors. Another peptide (P4) was recognized by 55% of sera from BK+ patients and by 5.5% of normal donors. The results presented here indicate the existence of T- and B-cell epitopes within the MTP40 protein. Given the particular recognition pattern of this protein, added to the fact that it appears to be a species-specific antigen of *M. tuberculosis,* a detailed study of the immune response to it may be useful in the design of more accurate diagnostic tests and an improved vaccine against human TBC.—Authors' Abstract

Ferreira, P., Soares, R. and Arala-Chaves, M. Susceptibility to infection with *Mycobacterium avium* is paradoxically correlated with increased synthesis of specific anti-bacterial antibodies. Int. Immunol. **3** (1991) 445–452.

A comparison was made between the levels of splenic and intestinal (Peyer's patches and thin intestinal epithelium) Ig production of C57BL/6 germ free and conventional C57BL/6, BALB/c, DBA/2 and C3H/He mice and the susceptibility to *Mycobacterium avium* infection, evaluated by the number of bacterial colony-forming units (CFU) found in the liver and in the spleen of the animals. Mice received an i.p. injection of either 5×10^6, 10^7 or 10^8 bacteria, or were given the larger inoculum intragastrically. Alternatively, mice were treated with an i.p. injection of *M. avium* bacterial sonicates. A marked increase of splenic IgA production, quantitatively associated with the size of the inoculum and thus with the degree of infection, was observed in susceptible compared to relatively resistant mice. This increase was observed at an earlier time following infection with the larger rather than with the smaller inocula. Consistent significant increases in splenic production of IgG isotypes were only observed in the susceptible mice after infection with the intermediate and larger inocula; whereas a comparative increase of IgM was only clearly observed after infection with the larger inoculum. Intestinal Ig production remained unchanged, however, in both susceptible and relatively resistant mice after i.p. infection. Also, all mice were resistant to *M. avium* infection by the intragastric route and with this site of entry splenic and intestinal Ig production remained unchanged. Susceptibility to *M. avium* infection was also quantitatively associated with increased levels of circulating specific antibacterial antibodies. That higher specific antibody responses observed in susceptible mice were not due to the increase of available antigenic material is shown by the fact that higher specific Ig production was also observed in the susceptible strains after injection of *M. avium* sonicates. Moreover, higher antibody response seems to be associated with susceptibility because the i.p. administration of serum containing specific Ig to resistant C3H/He strains increased the number of splenic bacterial CFU recovered at day 15 following i.p. infection with 10^8 *M. avium* by a factor of almost ten.—Authors' Abstract

Fujiwara, T. Synthesis of the trisaccharide-protein conjugate of the phenolic glycolipid of *Mycobacterium tuberculosis* for the serodiagnosis of tuberculosis. Agric. Biol. Chem. **58** (1991) 2123–2128.

The trisaccharide segment of the phenolic glycolipid (PGL) of *Mycobacterium tuberculosis,* 2-*O*-methyl-3-*O*-[3-*O*-(2,3,4-tri-*O*-methyl-α-L-fucopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranose, was synthesized in the form of the *p*-(2-methoxycarbonylethyl)phenyl glycoside by a stepwise condensation. 2,4-Di-*O*-benzyl-3-*O*-acetyl-α-L-rhamnopyranosyl chloride was coupled to *p*-(2-methoxycarbonylethyl)phenyl 4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranoside in the presence of silver triflate, and 2,3,4-tri-*O*-methyl-α-L-rhamnopyranosyl chloride was then coupled to the deacetylated disaccharide by the same procedure. The trisaccharide was deblocked and coupled to BSA, giving the neoglycoconjugate TB-NT-P-BSA. TB-NT-P-BSA

showed its possibility as a useful tool for the serodiagnosis of tuberculosis.—Authors' Abstract

Gordin, F. M., Perez-Stable, E. J., Reid, M., Schecter, G., Cosgriff, L., Flaherty, D. and Hopewell, P. C. Stability of positive tuberculin tests: are boosted reactions valid? Am. Rev. Respir. Dis. **144** (1991) 560–563.

To determine the stability and presumed significance of tuberculin skin tests, we followed a cohort of 380 tuberculin-positive patients living in chronic care facilities. Each patient had a positive reaction (≥ 10 mm induration to 5 tuberculin units of purified protein derivative) to one of three sequential baseline tuberculin tests. One year after the initial series, each patient had a single repeat skin test. Reversion to a negative test occurred in 98 (26%) of the 380 patients. Decreases in induration of 6 mm or more occurred in 88 (90%) of the reverters. Initially positive tests were more likely ($p < 0.001$) to remain stable than tests that were "boosted" to positive reactions on the second or third initial administration. Stable responses were found in 96% of those whose tests had ≥ 15 mm induration compared with 61% of those with reactions of 10 to 14 mm induration. Increasing age also was associated with a high rate of reversion. The instability of boosted tuberculin reactions brings into question the clinical significance of these tests. We propose limiting tuberculin testing to two sequential tests.—Authors' Summary

Heney, D., Norfolk, D. R, Wheeldon, J., Bailey, C. C., Lewis, I. J. and Barnard, D. L. Thalidomide treatment for chronic graft-versus-host disease. Br. J. Haematol. **78** (1991) 23–27.

The treatment of chronic graft-versus-host disease (GVHD) may present a difficult therapeutic problem. We used thalidomide to treat six patients with severe chronic GVHD who failed to respond to standard immunosuppressive agents. Four of the six patients showed a clear response to thalidomide, with the fifth patient showing a partial response. The best results were seen in patients with chronic cutaneous GVHD. Two of the patients developed neurophysiological evidence of a peripheral neuropathy, associated with clinical signs in one patient. Measurement of thalidomide plasma levels and pharmacokinetic curves showed a significant inter-patient variation. Peak plasma levels varied from 0.47 to 1.46 μg/ml. Thalidomide has a role to play in the management of chronic GVHD and further studies are needed.—Authors' Summary

Horsburgh, C. R., Jr., Havlik, J. A., Ellis, D. A., Kennedy, E., Fann, S. A., Dubois, R. E. and Thompson, S. E. Survival of patients with acquired immune deficiency syndrome and disseminated *Mycobacterium avium* complex infection with and without antimycobacterial chemotherapy. Am. Rev. Respir. Dis. **144** (1991) 557–559.

The contribution of disseminated *Mycobacterium avium* complex (DMAC) infection to the morbidity and mortality of patients with acquired immune deficiency syndrome (AIDS) is unclear. Previous studies that suggested the decreased survival of patients with AIDS and DMAC had incomplete information on patient immunologic status and follow-up. We studied patients with AIDS and DMAC and compared their survival with that of AIDS patients without DMAC but with other comparable risk factors for survival. Case and control subjects were similar in terms of CD4 cell count, prior AIDS status, history of antiretroviral therapy, history of *Pneumocystis carinii* prophylaxis, and year of diagnosis. A group of 39 patients with untreated DMAC had significantly shorter survival, mean of 5.6 ± 1.1 months (median 4 months), than 39 matched patients with AIDS but without DMAC, mean 10.8 ± 1.3 months (median 11 months, $p < 0.0001$). The survival of 16 additional patients with DMAC who received antimycobacterial therapy, mean of 9.5 ± 1.4 months (median 8 months), was not significantly shorter than that of an additional 16 matched control subjects, mean 11.7 ± 1.9 months (median 11 months, $p = 0.58$). Patients with treated DMAC survived significantly longer than those with untreated DMAC ($p < 0.01$). We conclude that untreated DMAC significantly shortens

survival. Moreover, these results indicate that patients with DMAC who receive antimycobacterial therapy do not experience the shortened survival seen in untreated DMAC.—Authors' Summary

Huang, Z. H., Ross, B. C. and Dwyer, B. Identification of *Mycobacterium kansasii* by DNA hybridization. J. Clin. Microbiol. **29** (1991) 2125–2129.

A DNA probe specific for *Mycobacterium kansasii* was obtained from a plasmid clone library of *Eco*RI-digested genomic DNA. The probe specifically identified culture-confirmed isolates of *M. kansasii* and isolates in cultures of environmental water samples. In an attempt to distinguish between isolates of *M. kansasii,* we used two methods to demonstrate restriction fragment length polymorphisms in the genomic DNA. Both of these methods failed to detect any differences between the isolates. These isolates included the type strain TMC 1201, environmental isolates, and clinical isolates from Australia and the Solomon Islands. This result suggests that the genome of *M. kansasii* is highly conserved and that genetic divergence within this species is insignificant.—Authors' Abstract

Izaki, S., Tanji, O., Okuma, M., Shimoda, H., Hsu-Oyama, N. P. S., Hibino, T. and Kitamura, K. IA antigen-positive epithelioid cells in experimentally induced granulomatous inflammation. J. Dermatolog. Sci. **2** (1991) 24–32.

IA antigens on the cell membrane of inflammatory macrophages and epithelioid cells were investigated with immunoelectron microscopic method during development of granulomas induced by subcutaneous inoculation of 10^7 *Mycobacterium lepraemurium* into mice with and without hypersensitivity. In C57BL/6N (H-2^b) immunogenetic high responder mice 6 weeks after infection majority (87%) of infiltrated cells were IA-positive. Two types of the staining reaction, strong and weak reactivity, were recognized among the positive cells. Strongly IA-positive cells showed lower phagocytosis (0.9/cell section) of mycobacteria than the weakly reacted cells (4.9/cell section). The strongly positive cells underwent morphological differentiation into large epithelioid cells during development of the hypersensitivity-type murine lepromas after 10 or more weeks of infection. Types of granulomas and IA-positive cells in C57BL/6N (nu/+) mice were identical to those found in C57BL/6N. In C57BL/6N (nu/nu) athymic nude mice initial infiltrating cells contained 38% of weakly IA-positive macrophages and a small number (7%) of strongly IA-positive macrophages. But the reactivity was lost later and only 4% of IA-positive cells remained in the granulomas without hypersensitivity. CBA/J (H-2^k) low responder mice did not show IA-positive cells in either initial or late stage during the development of nonhypersensitivity-type murine lepromas. We suggest that the presence of IA-positive cells, particularly IA-positive epithelioid cells, in the lesions modulates the course of granulomatous tissue reaction in murine lepromas.—Authors' Abstract

Ji, B., Truffot-Pernot, C. and Grosset, J. *In vitro* and *in vivo* activities of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis.* Tubercle **72** (1991) 181–186.

The *in vitro* and *in vivo* activities of sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* are reported. The MICs of sparfloxacin for 50% and 90% of 18 clinical isolates were, respectively, 0.25 and 0.5 mg/l, one or two dilutions lower than that of ciprofloxacin and ofloxacin. In mice infected intravenously with 0.1 mg *M. tuberculosis* H37Rv strain, the minimal effective dosage of sparfloxacin, as assessed by survival rate, spleen enlargement and gross lung lesions, was 12.5 mg/kg. The activities of various regimens were in the following rank order: INH 25 mg/kg = sparfloxacin 50–100 mg/kg > ofloxacin 300 mg/kg > (or =) sparfloxacin 25 mg/kg > sparfloxacin 12.5 mg/kg > (or =) ofloxacin 200 mg/kg > ofloxacin 100 mg/kg > (or =) negative control. Therefore, on a weight-to-weight basis, sparfloxacin was six- to eightfold more active against *M. tuberculosis* infection in mice than ofloxacin. In addition, WIN 57273, a new broad-spectrum fluoroquinolone, at a dosage of 100 mg/kg daily, was inactive against

M. tuberculosis infection.—Authors' Summary

Kucherov, A. L. [Antituberculosis care organization under conditions of a new economic system.] Probl. Tuberk. **6** (1991) 5–8. (in Russian)

The organizational forms of antituberculosis care now applied fail to promote the achievement of better final results at minimal expenses of material and personnel resources. New principles of financing of the antituberculosis service are required as well as the assessment criteria of its activity and changes in the antituberculosis aid tactics. The principle of separate financing of long-term tuberculosis–related programs opens perspectives for the improvement of the organizational forms of antituberculosis aid. It is required that the preventative antituberculosis measures exercised among the whole population be conducted in groups of population subjected to a high risk of tuberculosis infection.—Author's English Abstract

Mugusi, F., Swai, A. B. M., Turner, S. J., Alberti, K. G. M. M. and McLarty, D. G. Hypoadrenalism in patients with pulmonary tuberculosis in Tanzania: an undiagnosed complication? Trans. R. Soc. Trop. Med. Hyg. **84** (1990) 849–851.

In Dar es Salaam, Tanzania, 50 consecutive patients with pulmonary tuberculosis had their adrenocortical function assessed with a standard Synacthen test: no patient had received rifampin (which increases cortisol catabolism) within 1 month of the test. Height, weight, body mass index (BMI) and blood pressure (supine and erect) were measured at the same time. Two patients had low baseline cortisol levels (of whom 1 had an abnormal Synacthen response). Fifteen (30%) had normal basal cortisol levels, but impaired responses to Synacthen. The 16 patients with impaired responses were similar to those with normal responses with respect to symptoms, duration of tuberculosis, and BMI. The mean supine and erect diastolic pressures were significantly lower in the impaired response group (64 and 62 mm Hg vs 74 and 73 mm Hg). Adrenal hypofunction is common in patients with pulmonary tuberculosis in Tanzania, and may have a significant effect on both outcome and response to treatment.—G. H. Rée (Trop. Dis. Bull.)

Noronha, D., Pallangyo, K. J., Ndosi, B. N., Lweno, H. and Sabuka, S. R. Radiological features of pulmonary tuberculosis in patients infected with human immunodeficiency virus. E. Afr. Med. J. **68** (1991) 210–215.

During April–June, 1988, at Muhimbili Medical Centre, Dar es Salaam, Tanzania, 120 consecutive patients with sputum-positive pulmonary tuberculosis (TB) were studied; 71 (59%) patients were male and 49 (41%) were female. HIV-1 seroprevalence in the 16–45 year age group was 53% for men and 26% for women. Atypical chest-radiograph findings were seen in 21 (38%) of the 43 seropositive cases compared with 15 (26%) of 57 HIV-seronegative control patients. Four patients had miliary TB, 3 of whom were HIV-1 seropositive. Findings typical of reactivation TB were seen in 22 (51%) of seropositive cases compared with 42 (73%) of control cases. [HIV serostatus was determined by ELISA without confirmation.]—H. Richardson (Trop. Dis. Bull.)

Ogawa, T., Uchida, H., Kusumoto, Y., Mori, Y., Yamamura, Y. and Hamada, S. Increase in tumor necrosis factor alpha- and interleukin-6-secreting cells in peripheral blood mononuclear cells from subjects infected with *Mycobacterium tuberculosis*. Infect. Immun. **59** (1991) 3021–3025.

We detected and quantified tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) from monocytes/macrophages (Mϕ) in the peripheral blood of subjects from three different population groups, i.e., tuberculin-negative healthy subjects, tuberculin-positive healthy subjects, and patients with active pulmonary tuberculosis. TNF-α or IL-6 activity in the culture supernatant of these cells was determined by the cytotoxicity of murine L-929 cells or by enzyme-linked immunosorbent assay, respectively. Detection and enumeration of cells secreting either TNF-α or IL-6 were performed by an adaptation of the enzyme-linked immunospot assay. Monocytes/Mϕ from tuberculin-positive healthy subjects or patients with tuberculosis showed higher

TNF-α- and IL-6-producing activities than those from tuberculin-negative healthy subjects. The number of TNF-α- and IL-6-secreting cells in either lipopolysaccharide- or muramyl dipeptide-stimulated mononuclear cells from tuberculin-positive healthy subjects and patients was significantly higher than that in cells from the tuberculin-negative healthy subjects.—Authors' Abstract

Sada, E., Brennan, P. J., Herrera, T. and Torres, M. Evaluation of lipoarabinomannan for the serological diagnosis of tuberculosis. J. Clin. Microbiol. **28** (1990) 2587–2590.

An ELISA is described that uses purified lipoarabinomannan from *Mycobacterium tuberculosis* as antigen. Screening of serum samples from tuberculosis patients in Mexico showed that the test is comparable in specificity (91%) and sensitivity (72%) to previously described tests using defined mycobacterial antigens. The authors point out, however, that a high level of false-positive results was obtained with sera from patients with pulmonary histoplasmosis, raising the possibility that *Histoplasma* may express a crossreacting antigen.—D. Young (Trop. Dis. Bull.)

Van Vooren, J. P., Drowart, A., De Cock, M., Van Onckelen, A., D'Hoop, M. H., Yernault, J. C., Valcke, C. and Huygen, K. Humoral immune response of tuberculous patients against the three components of the *Mycobacterium bovis* BCG 85 complex separated by isolectric focusing. J. Clin. Microbiol. **29** (1991) 2348–2350.

An isoelectric-focusing technique followed by Western blot (immunoblot) analysis was used to investigate the immunoglobulin G response of tuberculous patients against each of the three components of the *Mycobacterium bovis* BCG antigen 85 complex. The 85A component was stained by the tuberculous as well as the nontuberculous sera. In contrast, the 85B and the 85C proteins of the complex were not stained by the control sera but were stained by 20 of 28 tuberculous serum samples.—Authors' Abstract