CURRENT LITERATURE

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

General and Historical

De Rojas, V. and del Rincon, K. [Level of knowledge of leprosy sufferers from the city of La Habana about their disease.] Rev. Cuba Med. Trop. **41** (1989) 64–75. (in Spanish)

The conclusions herein reported were derived from a study of the perceptions of the general public on leprosy compared with the concepts held by patients. This paper is based on a survey developed accordingly, which was applied to 150 patients and 100 healthy individuals in Havana City. Results show the need for increasing the health education provided patients.—Authors' English Summary

Ma, H., Ye, G.-Y., Shu, H.-W., Jiang, C. and Zhou, D.-S. Studies on social medicine and leprosy in east China. Proc. CAMS and PUMC **4** (1989) 61–64.

Leprosy is a disease in which the social, economic, cultural and environmental factors play an important role in occurrence, spread, control, eradication and rehabilitation. Eliminating the unfavorable factors hindering leprosy control can make achieving the goal of basic eradication of leprosy in China before the end of this century possible. For this reason, since 1984 we have carried out systematic studies of the social aspects of leprosy in Baoying County, a former high-endemic area in Jiangsu Province of eastern China, where the endemicity of leprosy is nearing full control.—Authors' Abstract

Max, E. Leprosy control in India: international cooperation. Chapter 13 in: *International Cooperation for Health; Problems, Prospects, and Priorities.* Reich, M. R. and Mariu, E., eds. Dover, Massachusetts: Auburn House Publishing Company, 1989, pp. 295–313.

The discussions in this chapter indicate that current approaches to the control of leprosy in India are in certain ways fundamentally wrong and that new approaches based on interdisciplinary and international cooperation are needed, some of them urgently, if the promise of modern medical technology for leprosy control is to be maintained.

Promotion of medicine for the benefit of patients and emphasis on effective application of economics and other social sciences in health care management are two main components of the medical philosophy advocated by Dr. Takemi. The foregoing discussions demonstrate the inevitability and importance of these components of Dr. Takemi's philosophy for the scientific management of leprosy control. Considerable scope exists for drawing on economics and other social sciences and for reforming them, by interdisciplinary and international cooperation, to promote collaborative and multidisciplinary research for providing a more rational basis for decision-making and programming activities in leprosy control.

This chapter recommends research and action to be undertaken on two main points. First, a sound understanding is needed of the process of physical impairments in leprosy and of the relevance and usefulness of family participation, telematics, and informatics for the prevention and control of these impairments. Second, efforts need to be made to develop an interdisciplinary framework linking the relevant disciplines to strengthen the conceptual basis and methodology for policy analysis and program planning to improve the efficiency of leprosy control.

Constructive cooperation between developed and developing countries can assist the process of making medical and social sciences serve their true objective of improv-

ing the quality of human life and of creating a welfare community of humankind.—Authors' Conclusion

Mull, J. D., Wood, C. S., Gans, L. P. and Mull, D. S. Culture and "compliance" among leprosy patients in Pakistan. Soc. Sci. Med. **29** (1989) 799–811.

In Pakistan approximately 30% of the 18,000 known leprosy patients have dropped out of their treatment programs. To investigate reasons for such widespread noncompliance, 128 diagnosed leprosy patients—59 outpatients and 69 inpatients—were interviewed in Karachi. More than half of the "noncompliant" outpatients denied having the disease. Denial was found to be an understandable coping mechanism in view of the severe stigma associated with leprosy. The presence of close-knit extended families, in which joint decision-making was the norm and in which such a dread diagnosis could spell the end of job and marriage prospects for even distant relatives, contributed to the likelihood of denial. In such a setting, the very term "noncompliant" appeared to be an oversimplification since it covered so many different types of culturally constrained behavior. In addition, many of the patients who initially seemed most "compliant" by virtue of being long-term hospital inpatients in fact owed their hospitalization to the fact that they had been markedly "noncompliant" in the past. Thus the usual view that adherence to a biomedical treatment regimen constitutes "compliance" and that nonadherence to such a regimen constitutes "noncompliance" proved inadequate for understanding the health behavior of these Third World leprosy victims. The study also showed that many patients had initially consulted traditional healers, inadequately trained physicians, and/or untrained medical practitioners for treatment of their symptoms, which resulted in lengthy delays before they were correctly diagnosed. Further, even after the diagnosis was made and appropriate medications were prescribed by trained personnel, most patients were not told what had caused their leprosy and how the drug regimen worked to combat it: when questioned, only 4% of the 128 respondents attributed the disease to infectious organisms. In addition, patients were usually not warned in advance of the possibility of undesirable side effects from their leprosy medications, which led to further "compliance" problems. The findings of this study emphasize the need for better training of physicians and other health care providers in early diagnosis of leprosy and better health education of diagnosed patients. To be truly effective, the treatment of leprosy must include counseling of extended families and education of the public at large as well as enhanced communication with the patients themselves.—Authors' Abstract

Volinn, I. J. Issues of definitions and their implications: AIDS and leprosy. Soc. Sci. Med. **29** (1989) 1157–1162.

It is demonstrated how definitions can determine social consequences of impairment and disability. A comparison between leprosy and AIDS provides the basis for the discussion. The United States is the geographic and political arena under consideration. Issues of classification as STD (sexually transmitted disease) or as contagious, communicable disease are relevant. An important factor to predict the social impact is the nomenclature utilized by CDC (Centers for Disease Control). CDC represents the government as the official agency to gather and report morbidity and mortality information. Hypotheses to explain stigma on the basis of epidemiological bases are added to the usual sociological concepts or historical considerations. Potential application of the findings are discussed.—Authors' Abstract

Chemotherapy

Carmichael, A. J. and Paul, C. J. Idiosyncratic dapsone induced manic depression. (Letter) Br. Med. J. **298** (1989) 1524.

Twenty-four hours after starting dapsone the patient had a two-day episode of hypomania, with flight of ideas, pressure of

speech, and marked hyperactivity, and lack of need of sleep. After three more days, she entered a depressive state, becoming withdrawn, low in affect, and tearful, with no change in her life circumstances. She gave no personal or family history of psychiatric problems and was taking no other medication. Her hemoglobin concentration was normal and methemoglobin was undetected. Dapsone was stopped after 10 days' treatment and within a fortnight her mood had returned to normal.—(*From the* Letter)

Dixit, V. B., Chaudhary, S. D. and Jain, V. K. Clofazimine induced nail changes. Indian J. Lepr. **61** (1989) 476–478.

Two cases of lepromatous leprosy with erythema nodosum leprosum, who were on high doses of clofazimine, showed discoloration of nail plate, subungual hyperkeratosis and onycholysis. These nail changes gradually disappeared when the dose of clofazimine was reduced.—Authors' Abstract

Franzblau, S. C. and White, K. E. Comparative in vitro activities of 20 fluoroquinolones against *Mycobacterium leprae.* Antimicrob. Agents Chemother. **34** (1990) 229–231.

The *in vitro* activities of 20 fluoroquinolones against *Mycobacterium leprae* were evaluated by using the BACTEC 460 system. *M. leprae* was incubated in BACTEC 12B medium at 33°C under reduced oxygen for 2 to 3 weeks in the presence of fluoroquinolones at 0.31 to 5 μg/ml. Activity was determined by a reduction in $^{14}CO_2$ evolution compared with that of drug-free controls. Of the commercially available agents, ofloxacin was most active, while enoxacin and norfloxacin were inactive. However, a number of newer fluoroquinolones (AT-4140, OPC-17100, OPC-17066, PD-117596, PD-124816, PD-127391, and WIN-57273), all containing a cyclopropyl group of R-1 and, with the exception of WIN-57273, either a halogen or methyl group at R-8, were more active than ofloxacin *in vitro.* Further *in vivo* evaluations of these agents should help determine their potential for use against leprosy.—Authors' Abstract

Gallo, M. E. N., Damasco, M. H. S., Alburquerque, E. C., Silva, T. C. P. G. and Almeida, S. M. R. [Secondary sulfone resistance related to a case of hanseniasis in Rio de Janeiro, Brazil.] Med. Cutan. Ibero Lat. Am. **17** (1989) 103–104. (in Portuguese)

We report on a case of dapsone resistance in a BL patient with clinical diagnosis and laboratory confirmation. The resistance appeared after 17 years of dapsone treatment, and the laboratory tests revealed total resistance to all the tested dapsone concentrations. The case reported occurred in the city of Rio de Janeiro (Brazil) which presents a leprosy prevalence of 1.79/1000.—Authors' English Summary

Gawkrodger, D. Manic depression induced by dapsone in patients with dermatitis herpetiformis. (Letter) Br. Med. J. **299** (1989) 860.

About 6 months after starting dapsone 50 mg daily a 35-year-old man with a 3-year history of dermatitis herpetiformis (proved by biopsy) developed symptoms clearly related to ingestion of dapsone. Within 2–3 hr of taking dapsone by mouth he developed headaches, had feelings of depression and anger, and became argumentative and upset. Without the dapsone he did not have these symptoms, but the eruption returned within 2 days despite his gluten-free diet. When he changed to sulfapyridine 500 mg 4 times a day these symptoms disappeared almost immediately, and after 6 months of sulfapyridine treatment he had had no recurrence. He had no history of psychiatric disorder.—(*From the* Letter)

Goloshchapova, E. N. [Immunocorrecting characteristics of new antileprosy drugs.] Vestnik. Dermatol. Venerol. **5** (1989) 7–9. (in Russian)

Antimycobacterial and immunotropic characteristics of new antileprosy drugs, made in this country, have been studied and compared with those of dapsone. Animal experiments have demonstrated a high immunostimulating activity of the new drugs; this calls for clinical trials of these agents, for they may improve the therapy efficacy, help prevent the disease recurrences, and

cut down the length of treatment for leprosy.—Author's English Summary

Kar, P. K. and Sohi, A. S. Study of multidrug therapy in paucibacillary leprosy. J. Indian Med. Assoc. **87** (1989) 34–36.

A study was undertaken to evaluate the efficacy of multidrug therapy (MDT) in paucibacillary leprosy. Out of 155 fresh cases studied 48 had indeterminate, 38 tuberculoid and 69 had borderline tuberculoid leprosy. Out of 155 cases, 64 patients in the first group were treated with dapsone 100 mg daily for 12 months. In the second group, 91 patients were given MDT, consisting of rifampin in 600 mg once a month and dapsone 100 mg daily for 12 consecutive months.

The cases receiving dapsone and the cases having MDT remained clinically active at the end of 6 months in 56.1% and 37.2%, respectively; 13.1% of the cases having single lesion and 66.3% of patients with multiple lesions were found to be active after 6 months of MDT. At the end of 1 year 79.6% of the cases receiving dapsone and 91.2% of the cases having MDT became inactive.—Authors' Abstract

Kenny, M. T., Torney, H. L. and Balistreri, F. J. Comparative effect of the naphthalenic ansamycins rifamycin SV, rifampin and cyclopentylrifampicin on murine neutrophil function. Int. J. Immunopharmacol. **11** (1989) 915–920.

The purpose of this study was to evaluate the *in vitro* effect of the naphthalenic ansamycins rifamycin SV, rifampin and cyclopentylrifampin on neutrophil degranulation and cytokinesis using murine peritoneal exudate cells. It was found that the FMLP-stimulated secretion of myeloperoxidase was significantly inhibited by 80 μg rifamycin SV ($p < 0.05$) and cyclopentylrifampin ($p < 0.01$) per ml. Nondirected and FMLP-directed migration was significantly ($p < 0.01$) inhibited by rifamycin SV and rifampin at assay concentrations above 0.31 and 5.0 μg/ml, respectively, thus confirming the low-dose rifamycin effect observed by others using human neutrophils. Finally, cyclopentylrifampin was shown to have no significant effect on nondirected or FMLP-directed neutrophil migration at assay concentrations of 1.25 to 80 μg per ml.—Authors' Abstract

Krishnan, S. Therapeutic response of modern chemotherapy in Hansenology. Chemioterapia **6** Suppl. 2 (1987) 358–359.

The latest regimen of treatment (WHO MDT) is accepted by the community, in spite of the low socioeconomic and educational status. The accessibility to all the clinics except one is good for both the medical team and the patients. The response to the drug therapy is remarkable without any noticeable side effects apart from one case due to Lamprene.—Author's Conclusion

Li, H.-Y., *et al.* [Observation on the therapeutic effect of short-term combined chemotherapy in multibacillary leprosy—review of 80 cases during the treatment and 33 months after treatment in Shandong and Yunnan Provinces.] Chin. J. Clin. Dermatol. **18** (1989) 286–289. (in Chinese)

Thirty-three active multibacillary (MB) patients from Shandong and 47 active MB patients from Yunnan were treated with 24 and 27 months of combined chemotherapy, respectively, and were followed up for 33 months by independent teams of these two provinces. The clinical, bacteriological, and histopathological evaluations were practically identical, and there were continued improvements after cessation of therapy. At 33 months after treatment, 34.2% (26/76) of patients had marked improvement and 65.8% (50/76) clinical cure.—Authors' English Abstract

Mariette, X., Mitjavila, M. T., Moulinie, J. P., Bussel, A., Brouet, J. C., Vainchenker, W. and Fermand, J. P. Rifampicin-induced pure red cell aplasia. Am. J. Med. **87** (1989) 459–460.

Pure red cell aplasia (PRCA) is characterized by severe anemia with reticulocytopenia in the absence of neutropenia or thrombocytopenia, and isolated erythroblastopenia in the bone narrow. It can occur as an idiopathic hematologic disorder or it may be associated with diverse conditions, including thymoma, lymphoid diseases, autoimmunity, and viral infections. In addi-

tion, PRCA may develop after exposure to drugs and has been attributed to antibiotics, especially isoniazid. We report herein the case of a patient treated with pefloxacin and rifampin in which *in vitro* studies suggested that rifampin induced PRCA through a drug-dependent serum inhibitor of erythroid colony formation.—(*From the* Article)

McDougall, A. C. and Georgiev, G. D. The chemotherapy of leprosy. Postgrad. Doctor—Africa **11** (1989) 93–99.

Included in this review article, which concentrates on multipledrug therapy (MDT), are four useful tables: the relationship of the MDT "multibacillary and paucibacillary" groups to the three clinical classifications, the details of the WHO recommended combined chemotherapy regimens, the pharmacology of the major antileprosy drugs (level of bactericidal activity, dosage, presentation, and shelf-life), and the main toxic side effects of antileprosy drugs.—C. A. Brown (Trop. Dis. Bull.)

Meng, M.-B., *et al.* [Examination of viability of *M. leprae* with FDA/EB staining in MDT.] China Lepr. J. **5** (1989) 129–131. (in Chinese)

A fluorescent staining procedure (FDA/EB) for determining the viability of *Mycobacterium leprae* was used in 75 multibacillary leprosy patients, who were being treated by WHO's MDT. The average percentage of green-stained *M. leprae* in 11 cases was 44.43% before treatment and in the cases treated for 4–6 months it decreased rapidly to 7.66%. Since then, it has been slowly decreasing; 42.86% of the cases still have green-staining bacilli in the group treated 19–24 months, in whom the average percentage of green-stained *M. leprae* was 2.62%. The fluorescent staining procedure (FDA/EB) can be used as one of the methods to judge the effect of MDT. The authors considered that the stopping of the MDT after 2 years in all the patients seems not to be suitable and then, if the patients still have green-stained bacilli, the MDT ought to be continued.—Authors' English Abstract

Orts Poveda, M. del C. and Bandrés Sánchez, M. P. [Application of WHO multidrug therapy in San Pablo (Peru).] Rev. Leprol. Fontilles **17** (1989) 251–257. (in Spanish)

The present work has been done in the former leprosy center of San Pablo (Peru). It shows our experience in administering multidrug therapy to 43 lepromatous leprosy patients, who were reported clinically and skin smear active at the beginning of the treatment. All the patients had 28.6 years average illness duration and had previously received dapsone monotherapy. They took 24 multidrug therapy doses until skin-smear negativity. As an average, the clinical improvement was achieved in 11.5 months, and the smear negativity was attained in 15.2 months. After the treatment conclusion, the supervision showed one woman's reactivation after pregnancy. There were no side effects obligating to interrupt the therapy.—Authors' English Summary

Sansarricq, H. Technical problems related to multidrug therapy in leprosy control. Acta Leprol. (Genève) **7** (1989) 59–62.

About 40% of registered leprosy patients worldwide have been, or are being, treated with multidrug therapy (MDT) in accordance with the standard regimens recommended by WHO in 1981. The conclusions that can be drawn from such an extensive experience are discussed. The most significant of these are: (i) the MDT regimens recommended by WHO are nontoxic and well accepted by patients; (ii) as regards the efficacy of these regimens, post-therapeutic relapses, in both multibacillary and paucibacillary patients, have been negligible when observed over periods of 1 to 3 years. The overall conclusion that at present emerges is that the MDT regimens for leprosy control, as recommended by WHO in 1981, should continue to be applied without any modification.—Author's Summary

Sengupta, A., Gupta, J. K., Dutta, J. and Ghosh, A. The component fatty acids of chaulmoogra oil. J. Sci. Food Agric. **24** (1973) 669–674.

The fatty acid composition of the seed oils of the species, *Hydnocarpus kurzii, H. wightiana* and *H. odorata* were determined by gas-liquid chromatographic (g.l.c.) analysis. The percentages of individual fatty

acids were found to be: hydnocarpic 23.0, 33.9,—; chaulmoogric 29.6, 35.0,—; gorlic 25.1, 12.8,—; lower cyclic homologues 0.3, 4.6,—; myristic 0.6, 0.8, 0.4; palmitic 8.4, 5.6, 11.8; stearic—, 0.6, 4.7; palmitoleic 6.0, 1.3, 0.5; oleic 5.4, 3.6, 21.8; linoleic 1.6, 1.8, 29.3; linoleic—, —, 31.2, respectively.—Authors' Abstract

Thakur, M., Vaishnavi, C., Ganguly, N. K., Kaur, S. and Kumar, B. Primary dapsone resistance as assessed by uptake of labelled thymidine by the macrophage resident *Mycobacterium leprae.* Indian J. Lepr. **61** (1989) 437–441.

Twelve untreated lepromatous leprosy patients were screened for primary dapsone resistance by the uptake of labelled thymidine by macrophage resident *Mycobacterium leprae.* Three were found to harbor primary dapsone-resistant strains of *M. leprae* and another three, partially resistant strains to the drug. This rapid, simple and reliable method should be used routinely to screen leprosy patients for drug resistance. —Authors' Abstract

Wang, H.-Y., *et al.* [Study on short-term clinical trial of R-77-3 [3-(4-cyclopentyl-1-piperazinyl) imino methyl rifamycin SV] by mouse foot-pad technique.] Chung Kuo I Hsueh Ko Hsueh Yuan Hsueh Pao **10** (1988) 374–375. (in Chinese)

R-77-3 (DL473, rifapentine) is a new semisynthetic antibiotic of rifamycin SV. In this paper, a short-term clinical trial of R-77-3 was studied by the model of mouse foot pad infected with *Mycobacterium leprae.* The antileprosy action of R-77-3 was observed in 7 cases of multibacillary leprosy patients with single dose of 400 mg (2 cases) or 600 mg (5 cases) under the supervision of the authors. *M. leprae* taken from patients were inoculated into mouse foot pads before and 3, 7, 14, 28 days after treatment. A preliminary results indicated that R-77-3 is a rapid bactericidal drug for *M. leprae.* Because the half-life of R-77-3 is longer than rifampin it might be better for the intermittent treatment of leprosy.—Authors' English Abstract

Zeng, X.-L., *et al.* [Effect of MDT in 52 cases of MB leprosy for one and a half years.] China Lepr. **5** (1989) 143–144. (in Chinese)

Fifty-two cases of multibacillary leprosy, including 27 LL, 18 BL, and 7 BB patients, have been treated with daily doses of 100 mg of dapsone (DDS) and 50 mg of clofazimine (B663) and monthly doses of 600 mg of rifampin (RFP) and 300 mg of B663 for 18 months, of which 12 cases, accounting for 23.70%, were cured or basically cured, 23 cases (44.24%) gained obvious improvement and 17 had improvement clinically. Their BI was 1.62 on an average before the treatment and, respectively, 1.52, 1.2 and 0.8 at 6, 12 and 18 months of the treatment, and parallel effect has been seen in pathological examination. Most symptoms of 12 ENL cases and 8 neuralgic cases have gradually subsided with the treatment. No serious side effect was seen during the treatment. The authors considered that the regimen could be used on a large scale.—Authors' English Abstract

Zhou, Y. [The color in the urine of leprosy patients taking MDT and their compliance.] China Lepr. J. **5** (1989) 196–198. (in Chinese)

The examinations of the color and optical density of the urine in 47 leprosy patients taking MDT show that no brown-red color was seen whether in the patients taking clofazimine (B663) over a long period of time or in those who took a single dose of 300 mg B663 and also there was no red-colored sweat, saliva, sputum, feces or hair, but 24 hours after taking a single dose of 600 mg rifampin the urine would become red. The author points out that excretion of B663 from the body is very slow and only its trace emerges in the urine, consequently the compliance of patients may not be judged from the color of their urine.—Author's English Abstract

Clinical Sciences

Clague, R. B., Morgan, K., Reynolds, I., Misra, H., Majumdar, V., Hazra, S. K. and Chaudhury, S. N. Lack of serum antibodies to native type II collagen in leprosy. (Letter) Ann. Rheum. Dis. **49** (1990) 67.

We obtained serum samples from 10 patients (9 male, 1 female, aged 23–60 years) with leprosy (7 lepromatous leprosy, 2 tuberculoid leprosy, and 1 borderline), all with joint involvement (both large and small joints in 5, small joints only in 3, and large joints only in 2). Five were receiving regular treatment, three had taken intermittent treatment, and two were untreated. Serum antibodies to native type II and denatured type II collagen were measured by enzyme-linked immunosorbent assay (ELISA). Serum samples from 22 normal controls were used to determine an upper limit of normal as three standard deviations above the mean. A known high positive control (RA) was included. None of our patients had raised serum antibodies to native type II collagen, though three patients had slightly raised levels to denatured type II collagen. . . . our negative results suggest that antibodies to native type II collagen are largely restricted to RA.—(*From the* Letter)

Clark, C. E. and Richardson, G. A. Inappropriate ADH secretion in a patient with leprosy. (Letter) Br. J. Hosp. Med. **42** (1989) 340.

We report a case of inappropriate antidiuretic hormone (ADH) secretion in a patient with leprosy. We do not believe this to have been previously documented. This syndrome has previously been described with several types of infection. However, a thorough search of the literature has failed to locate any previous association with leprosy, neither is it a known side effect of any of the drugs mentioned, which had all been taken by the patient for several months before admission. While the serum osmolality is known to decrease during pregnancy, this is not an adequate explanation of the findings. We believe this is the first documented case of the syndrome of inappropriate ADH secretion in a pregnant woman suffering from erythema nodosum leprosum.—(*From the* Letter)

Dayal, R., Paliwal, A. K., Prasad, R., Mathur, P. P., Bharadwaj, V. P., Girdhar, B. K. and Pandey, D. N. A clinico-bacteriological profile of leprosy in children. Indian Pediatr. **26** (1989) 122–128.

Clinico-bacteriological profile of 106 leprosy patients below 15 years of age was studied. Majority of the patients were males and fell in the 10–15 years age group. Nearly 89% had not received any prior treatment because of financial constraints. Seventy percent gave a positive history of contact with adult patients who were mainly of the lepromatous variety. Skin lesions were present in 103 cases, mainly on the exposed areas, and their number was found to increase significantly with advancing age. These lesions were hypopigmented patches in 71% of the children and erythematous in the rest. Cutaneous sensations were affected in most of the patients while nerve thickening was observed in 45. Positivity of the skin smears increased significantly as the number of skin lesions per patient increased. With advancing age, the disease moved from the tuberculoid end of the spectrum towards the lepromatous end.—Authors' Abstract

ffytche, T. Blindness in leprosy—a forgotten complication. Aust. N.Z. J. Ophthalmol. **17** (1989) 257–260.

Leprosy remains one of the world's major blinding diseases and yet few ophthalmologists are aware of the spectrum of ocular complications. Cross-sectional studies of the eye changes in leprosy patients, made under standardized conditions, have been carried out in 24 different leprosy centers throughout the world and the preliminary results are presented. They show that up to 20% of leprosy patients develop sight-threatening lesions and between 5% and 7% are blind (depending on the definition of blindness).

Visual impairment in leprosy needs special consideration by leprologists and ophthalmologists, not only because much of it is preventable, but also because it is a severe burden to be added to the problems of mobility and social stigma that characterize this ancient disease.—Author's Abstract

Garg, R., Agarwal, J. K., Singh, G. and Bajpai, H. S. Hormone profile in leprosy. Indian J. Lepr. **61** (1989) 428–431.

Hormone profiles were carried out in 35 male cases of leprosy. They were divided into tuberculoid leprosy, borderline leprosy, lepromatous leprosy and lepra reaction. Serum testosterone, follicle-stimulating hormone, leutinizing hormone, and estradiol level were measured in these cases of leprosy. It was observed that serum testosterone levels were significantly low in lepromatous leprosy ($p < 0.001$) and lepra reaction ($p < 0.01$). The serum levels of follicle-stimulating hormone and leutinizing hormone were significantly high in lepromatous leprosy ($p < 0.02$) and lepra reaction ($p < 0.05$). Serum estradiol was raised in approximately 60% of cases in borderline leprosy, lepromatous leprosy and lepra reaction.—Authors' Abstract

Guimarães Proenca, N. [The interpretation of the Mitsuda and Kveim tests in the differential diagnosis between tuberculoid leprosy and cutaneous sarcoidosis.] Med. Cut. Ibero Lat. Am. **17** (1989) 163–165. (in Portuguese)

Patients with sarcoidosis that present only cutaneous lesions are uncommon but have been described. In countries where leprosy occurs as an endemic disease the differential diagnosis between sarcoidosis and tuberculoid leprosy may be difficult to establish. In order to arrive at the correct diagnosis this study presents a table in which the results of lepromin and Kveim tests are analyzed and correlated to the other.—Author's English Summary

Kazantseva, I. A., Belskaya, O. B. and Biryukov, A. V. [A case of lepromatous lepra.] Arkiv. Patologii **51** (1989) 72–74. (in Russian)

The paper describes a case of lepromatous leprosy diagnosed from the skin biopsy specimen taken in a 23-year-old male patient. It provides histologic signs that are beneficial for the correct diagnosis. Emphasis is placed upon the necessity for staining by the Ziehl-Neelsen method to detect microorganisms in infiltrative and granulomatous skin lesions.—Authors' English Summary

Kulkarni, V., Gharpuray, M. B. and Kulkarni, D. S. Multiple cold abscesses in a borderline lepromatous patient on multidrug therapy. Indian J. Lepr. **61** (1989) 442–444.

A 25-year-old male patient was diagnosed as a case of borderline lepromatous (BL) leprosy in erythema nodosum leprosum reaction. He was put on multidrug treatment. He took regular treatment. Approximately a year after the beginning of the treatment, he developed multiple cold abscesses and later tuberculosis of the left hip joint. He was given antitubercular treatment with four drugs and the abscesses were treated surgically. He showed good response. This unusual case and the role of intermittent rifampin is discussed.—Authors' Abstract

Lewallen, S., Courtright, P. and Lee, H.-S. Ocular autonomic dysfunction and intraocular pressure in leprosy. Br. J. Ophthalmol. **73** (1989) 946–949.

We examined 241 leprosy patients and 135 age-matched healthy controls in central South Korea, measuring intraocular pressure in the supine and the upright positions and measuring the size of the pupils in darkness as an indication of ocular autonomic dysfunction. The mean intraocular pressure was significantly lower in the patients and the mean size of pupils was significantly smaller in the patients than in the controls. However, there was no correlation between pupil size and intraocular pressure in our patients. Our findings show that leprosy patients have ocular autonomic dysfunction, but do not support previous speculation that this dysfunction is the primary cause for low intraocular pressure in leprosy.—Authors' Summary

Markusse, H. M., Smelt, A. H. M. and Teepe, R. G. C. Unusual arthritis: be on the alert for leprosy. Clin. Rheumatol. **8** (1989) 266–268.

A 50-year-old Indonesian man presented with arthritis of the left ankle, wrist and hand joints and a diffusely swollen left hand and foot. A few months later granulomatous skin lesions developed and renewed physical examination revealed a paresis of the intrinsic muscles of the left hand and the left M. extensor hallucis longus and thickening of several peripheral nerves. The skin lesions appeared to be anesthetic. A diagnosis of borderline tuberculoid leprosy was made and treatment resulted in cure with permanent peripheral nerve damage. This case emphasizes the importance of early recognition and treatment of leprosy to prevent nerve damage.—Authors' Summary

Mohamed, K. N. Dermatological disorders resembling leprosy. Singapore Med. J. **30** (1989) 265–269.

Three dermatological conditions—epidermolysis bullosa dystrophica (EBD), granuloma multiforme (GM) and mycosis fungoides (MF)—were diagnosed elsewhere as leprosy, either clinically or histologically. Although the morphology of the lesions were suspicious of leprosy there were few striking clinical findings which were unfavorable. Leprosy is still an important disease that should not be missed. However, the recognition of these skin disorders is highlighted so that unnecessary and prolonged treatment for leprosy can be avoided in endemic countries.—Author's Abstract

Patki, A. H. and Mehta, J. M. Pterygium unguis in a patient with recurrent type 2 lepra reaction. Cutis **44** (1989) 311–312.

A 35-year-old man with long-standing lepromatous leprosy and history of recurrent, severe type 2 lepra reaction was found to have pterygium unguis and destruction of the fingernails. We propose that the obliterative angiitis and endarteritis due to severe type 2 lepra reaction were responsible for these nail changes.—Authors' Abstract

Satti, M. B., Al-Mohaya, S. and Omer, A. S. Hansen's disease: a cause of lymphadenopathy in endemic areas. Trop. Geogr. Med. **41** (1989) 80–84.

In this paper we report two unusual cases of leprosy, both presenting with lymphadenopathy as the initial manifestation of the disease. Lymphadenopathy was the dominant presenting complaint of the first patient in whom skin lesions were absent. A diagnosis of lepromatous leprosy was made only after a lymph-node biopsy. Following this diagnosis neural involvement was evident. In the second patient the lymphadenopathy was associated with polyarthritis leading to a false clinical diagnosis of Still's disease. This unusual presentation in both cases led to a delay in the final diagnosis which was based on histopathological examination of lymph nodes. The clinical and histopathological features of both patients are discussed. Superficial nerves should be palpated in all patients presenting with lymphadenopathy in endemic areas.—Authors' Abstract

Sehgal, V. N. and Joginder. Tuberculoid (TT) leprosy: localization on a tattoo. (Letter) Lepr. Rev. **60** (1989) 241–242.

One year after being tattooed on the right wrist the woman had noticed a small erythematous patch over and around the tattoo. The patch had not developed further until about 15 years later when she presented with impaired sensation in the (enlarged) patch and in a similar eruption on the right index finger. From histological examination and skin tests the case was diagnosed as tuberculoid leprosy.—C. A. Brown (Trop. Dis. Bull.)

Sen, R., Sehgal, P. K., Singh, U., Yadav, M. S., Chaudhary, S. D. and Sikka, R. Bacillaemia and bone marrow involvement in leprosy. Indian J. Lepr. **61** (1989) 445–452.

Fifty patients (24 new and 26 receiving specific treatment) of leprosy were investigated to study the concentration and morphological index (MI) of the lepra bacilli in skin, peripheral blood and bone marrow. The organisms were detected in 28 cases on

slit-skin smear examination, in 38 cases on bone marrow examination, and in 38 cases on examination of smears made from the buffy coat of peripheral blood. Out of 22 cases negative for the bacilli on slit-skin smears, 15 had the organisms either in buffy coat or bone marrow or both. Acid-fast bacilli in peripheral blood and bone marrow with skin-smear negativity were mainly observed in patients with the paucibacillary type of the disease and in those who were receiving treatment. Examination of buffy coat and bone marrow for the presence of lepra bacilli is suggested to establish the diagnosis in doubtful cases.—Authors' Abstract

Shao, K.-W., *et al.* [Analysis of childhood leprosy in Fujian.] China Lepr. J. **5** (1989) 182–184. (in Chinese)

Among 3299 children with leprosy detected in Fujian Province, most of the first skin lesions are on the lower extremities (37.4%) followed by the upper extremities (25.1%). In the multibacillary forms, most of the first lesions are erythema or infiltration on the face (30.1%), and in the paucibacillary forms they are erythema, depigmentation and plaque. The incidence of leprosy in children is parallel with the endemic grade of leprosy in the population, i.e., the incidence rate in the children is in positive correlation with the incidence and prevalence of leprosy at the given place. When paucibacillary leprosy in the children is detected, the patients with only a single skin lesion account for 32.5% and those with a duration of the disease less than 2 years are in 42.95%, both higher than those in the adult. The rate of deformity in children is lower, which shows that childhood leprosy is easily detected in its earlier stage. The time needed for treatment until cure averages 86 months in the multibacillary and 54 months in the paucibacillary form; the relapse rate in these is 1.8%.—Authors' English Abstract

Soni, N. K. Antroscopic study of the maxillary antrum in lepromatous leprosy. J. Laryngol. Otol. **103** (1989) 502–503.

The technique of antroscopy affords an accurate assessment of chronic sinus disease. It was performed in 12 patients suffering from lepromatous leprosy in order to determine the type, nature and site of the lesion. Antral involvement with lepromatous leprosy may begin with a localized area of erythema progressing to granuloma formation or ulceration. The antero-inferior part of the antrum is the most commonly affected site.—Author's Abstract

Valayer, P. and Strobel, M. [Difficulties to detect Hansen's bacilli in lesions; a study of one case.] Med. Trop. **49** (1989) 305–306. (in French)

In one case of leprosy, the classic Ziehl-Neelsen staining technique could not present the bacteriological evidence. After using a cold-staining procedure, the Lapeyssonie and Causse technique, numerous Hansen's bacilli were detected in the lesions.—Authors' English Summary

Wei, G.-R., *et al.* [Histoid leproma is not rare—report on eight cases.] China Lepr. **5** (1989) 145–146. (in Chinese)

The histoid leproma was rarely reported in China. The authors have three cases of histoid leproma in an epidemiological survey in 1985, accounting for 9% (3/33) of active patients in the county surveyed and for 15% (3/18) of multibacillary cases. In addition, five cases of histoid leproma were at the skin clinic in 1986 to 1987, making up 16% (5/32) of new cases of leprosy and 28% (5/18) of multibacillary patients. On this basis, the authors point out that histoid leproma might not be very rare in Guizhou Province.—Authors' English Abstract

Yvonnet, B., Vincelot, P., Millan, J., Lesage, G., Denis, F., Languillon, J., Mboup, S., Coursaget, P., Diop Mar, I. and Chiron, J. P. Hepatitis B virus infection in lepromatous and tuberculoid patients from Senegal. Acta Leprol. (Genève) 7 (1989) 63–66.

Hepatitis B virus (HBV) seric markers (HBs Ag, anti-HBs, and HBe Ag) were studied in 987 Senegalese leprosy patients (lepromatous: LL; tuberculoid: TT) in comparison with 6187 healthy adults (controls).

Two populations of leprosy patients from ILAD (Institut de Léprologie Appliquée de Dakar) were studied: The first study (study I) between 1973 and 1977 included 553 patients (329 LL; 224 TT). The second study (study II) between 1982 and 1986 included 434 patients (236 LL; 198 TT). HBV serological markers were tested by various techniques. By RIA, they were present in 98% and 96.5% in the studies I and II, respectively. Each marker was studied and compared to the control population. HBs Ag detected by RIA was present in 25.5% (study I) and 23.0% (study II) compared to 15.2% in the control group. This marker was correlated with leprosy forms (LL and TT), age, sex, and ethnic group.—Authors' Summary

Immuno-Pathology

Ayed, K. [Immunological aspects of leprosy.] Acta Leprol. (Genève) **7** (1989) 37–40. (in French)

Lepromatous leprosy is accompanied by a complex deficit in cell-mediated immunity concerning *Mycobacterium leprae.* The physiological mechanism of this deficit remains unknown. According to some studies there may be, in those suffering from leprosy, trouble in the macrophage and presentation of antigens. Other studies suggest an increase in the suppressive activity of lymphocytes. Recently, some authors emphasized the deficit in the production of interleukin 2. It is difficult, for the time being, to find out whether such immunological abnormalities are primary or secondary to the accumulation of the bacillus into the organism.—Author's English Summary

Bharadwaj, V. P. and Katoch, K. Detection of subclinical infection in leprosy: an 8 years follow-up study. Indian J. Lepr. **51** (1989) 495–501.

A follow-up study has been carried out using fluorescent leprosy antibody absorption (FLA-ABS) test in 1069 healthy contacts of multibacillary and paucibacillary leprosy patients. Simultaneously, lepromin testing with Dharmendra antigen has also been done to determine their delayed-type hypersensitivity. In nearly 8 years of follow-up, 46 contacts have developed disease and of these 41 contacts were FLA-ABS positive and lepromin negative. It is inferred that this test (along with lepromin) can be used to identify the contacts who are at higher risk of developing the disease. The FLA-ABS test has also been found to be highly sensitive for detection of subclinical infection, especially in younger age groups. This test could therefore serve as a very sensitive epidemiological tool for assessing the extent of disease in the community and for monitoring the transmission of disease, especially after MDT and other intervention measures.—Authors' Abstract

Billingham, M. E. J., Carney, S., Butler, R. and Colston, M. J. A mycobacterial 65-kD heat shock protein induces antigen-specific suppression of adjuvant arthritis, but is not itself arthritogenic. J. Exp. Med. **171** (1990) 339–344.

A recombinant (r)65-kDa protein from *Mycobacterium leprae,* at levels far in excess of those present in whole mycobacteria, was unable to induce arthritis. Even when combined with a synthetic adjuvant, CP20961, to mimic the peptidoglycan adjuvant component of the mycobacterial cell wall, the r65-kDa protein failed to induce arthritis. Pretreatment with as little as 1 μg r65-kDa protein protected rats against arthritis induced by *M. tuberculosis,* but this r65-kDa protein was markedly less able to protect against arthritis induced by the synthetic adjuvant, CP20961, or type II collagen. The r65-kDa protein appears, therefore, to produce an antigen-specific protection against arthritis induced by bacterial cell walls containing the 65-kDa protein. Such protection can be overcome, however, by arthritogenic T lymphocytes, suggesting that protection occurs by preventing clonal proliferation of autoreactive T lymphocytes that are in-

duced by the adjuvant properties of mycobacterial cell walls. How the r65-kDa protein abrogates this particular adjuvant activity, and the nature of the arthritogenic self antigen(s), remain to be elucidated.—Authors' Summary

Bloom, B. R., Salgame, P., Vehra, V., Kato, H., Modlin, R., Rea, T., Brennan, P., Convit, J., Lugozi, L., Snapper, S. and Jacobs, W. Vaccine development; on relating immunology to the Third World: some studies on leprosy. Immunology Suppl. 2 (1989) 87–90.

Leprosy is of interest to immunologists because the varied clinical manifestations of the disease correlate closely with the immunological spectrum. Resistance to infection is dependent on appropriate cell-mediated immunity, but patients with the lepromatous form fail to respond to antigens of *Mycobacterium leprae. In vitro* studies have revealed the existence of T-suppressor cells of the phenotype CD8+, CD3+, HLA-DR+, FcR+, 9.3−, which are restricted by major histocompatibility complex (MHC) class II antigens. Several new candidate vaccines against leprosy have been effective in breaking immunological unresponsiveness and engendering cell-mediated immunity in lepromatous leprosy patients, including the combination of BCG + killed *M. leprae.* Because BCG has unique adjuvant properties, we have begun to use molecular genetic approaches to develop BCG into a multivaccine vehicle capable of immunizing simultaneously against several pathogens. Both phage-based and plasmid-based strategies have been successfully developed for introducing selectable markers into BCG for the first time.—Authors' Summary

Cologlu, A. S. Process of disintegration and degradation of *M. leprae*: study of tissue imprints and tissues. Indian J. Lepr. **61** (1989) 485–494.

The existence, distribution and behavior of degradation products of *Mycobacterium leprae* in leprosy lesions were investigated in tissue specimens fixed in neutral formalin and embedded in paraffin. Cytopathologic findings using tissue imprints were unsatisfactory. Sections were stained with hematoxylin-eosin, acid-fast stains, silver methenamine and by an immunochemical (PAP) technique using serial paraffin sections. A comparison in respect of the distribution of the bacilli within the macrophages showed considerable differences between the superficial and deep granulomas. This corresponds roughly with the central, intermediary and peripheral locations. In a small granuloma seen in BL lesions, there were two zones: central and peripheral. In a large LL granuloma, three zones were seen: central, intermediary and peripheral zones. It is suggested that the degradation of disintegrated particles of bacilli might be due to the lysozymal activity of macrophages. The phagocytized bacilli are slowly degraded with long incubation periods, but the undigested debris remains inside the phagosomes. The chemical complexity of cytoplasm, cell wall and lipid fractions of *M. leprae,* and it is such that the lipid fractions of *M. leprae* mask some other antigenic components, which may be responsible for the cellular response and lysozymal production. According to our findings we believe that chemotherapy kills *M. leprae* but degraded products are not removed. These components are chemically complex and digested with difficulty. Lysozymal enzymes could be inhibited from productions by the bacterial debris or the lipid fractions could serve as a mask to delay lysozymal production in the cell. These aspects need further study.—Author's Abstract

Fournie, J.-J., Adams, E., Mullins, R. J. and Basten, A. Inhibition of human lymphoproliferative responses by mycobacterial phenolic glycolipids. Infect. Immun. **57** (1989) 3653–3659.

The effect of mycobacterial phenolic glycolipids from *Mycobacterium leprae, M. bovis* BCG, and *M. kansasii* on *in vitro* proliferative responses by human blood mononuclear cells from healthy BCG vaccines was investigated. All three phenolic glycolipids inhibited proliferation in a concentration-dependent manner. Inhibition was independent of the stimulus used and involved neither antigen-presenting cells nor antigen-

specific CD8+ suppressor-T cells. It was concluded that the phenomenon may be a general property of mycobacterial phenolic glycolipids, perhaps analogous to the growth-modulating properties of gangliosides. Despite the lack of specificity of inhibition *in vitro, de facto* specificity may occur *in vivo* by virtue of the localization of glycolipid in the leprosy lesions.—Authors' Abstract

Gaston, J. S. H., Life, P. F., Bailey, L. C. and Bacon, P. A. In vitro responses to a 65-kilodalton mycobacterial protein by synovial T cells from inflammatory arthritis patients. J. Immunol. **143** (1989) 2494–2500.

Bacterial antigens (Ag), especially those of mycobacteria, have been implicated in the pathogenesis of experimental inflammatory arthritis in rodents, while in man, reactive arthritis has a clear temporal relationship to infection with particular bacteria. To investigate the role of immune responses to bacterial Ag in inflammatory arthritis, we have examined the proliferative responses of paired synovial fluid and PBMC when stimulated with 1) suspensions of irradiated or heat-killed bacteria associated with reactive arthritis (ReA), 2) purified protein derivative, 3) a recombinant 65-kDa heat-shock protein of *Mycobacterium leprae.* The 65-kDa Ag was stimulatory to synovial fluid mononuclear cells, but not PBMC, from patients with different arthropathies, including most of those with ReA, but also some with rheumatoid arthritis. Furthermore, the magnitude of these responses correlated more closely with responses to ReA-associated bacteria (such as *Salmonella*), than with responses to the mycobacterial Ag represented in purified protein derivative. These results suggest that the 65-kDa molecule, which is common to a wide range of bacteria, may be an important immunogen for the T-cell-mediated immune responses within the joint in different clinically defined inflammatory arthropathies.—Authors' Abstract

Gelber, R. H., Brennan, P. J., Hunter, S. W., Munn, M. W., Monson, J. M., Murray, L. P., Siu, P., Tsang, M., Engleman, E. G. and Mohagheghpour, N. Effective vaccination of mice against leprosy bacilli with subunits of *Mycobacterium leprae.* Infect. Immun. **58** (1990) 711–718.

Model vaccines against leprosy bacilli have consisted of nonvirulent, live, attenuated *Mycobacterium bovis* BCG and irradiated, heat-killed, or autoclaved intact *M. leprae.* We report that immunization with various cell wall fractions of *M. leprae,* progressively depleted of lipids, carbohydrates, and soluble proteins, as well as a partially purified protein(s) derived from a pellet fraction of sonicated *M. leprae,* conferred significant protection against subsequent infection with live leprosy bacilli. Moreover, lymphocytes from regional lymph nodes and spleens of mice immunized with these *M. leprae*-derived subunits responded by proliferation when stimulated with *M. leprae in vitro.* Our results provide the first evidence that vaccination with *M. leprae*-derived fractions protects mice against leprosy bacilli.—Authors' Abstract

Gill, H. K., Ridley, D. S., Ganesan, J., Mustafa, A. S., Rees, R. J. W. and Godal, T. *Mycobacterium leprae* reactive T cell clones from lepromatous leprosy patients after prolonged dapsone chemotherapy. Lepr. Rev. **61** (1990) 25–31.

The proliferative responses of peripheral blood mononuclear cells (PBMC) to *Mycobacterium leprae* and BCG were studied in two groups of leprosy patients: a group of 8 lepromatous patients who had been on treatment for more than 20 years (TLL) and a group of 8 untreated lepromatous leprosy patients (ULL). The mean response to *M. leprae* of the TLL group was 6195 cpm, with 5 of the 8 patients responding positively. The mean response to *M. leprae* of the ULL group was 617 cpm, with only 1 patient showing a positive response. The corresponding proliferative responses to BCG were 19,908 cpm in the TLL group and 7908 in the ULL group. Thirteen *M. leprae*-reactive clones were established from 2 TLL patients and 5 *M. leprae* reactive clones were established from 2 tuberculoid leprosy patients. Seven of these clones, 4 from the TLL patients and 3 from the tuberculoid (TT) patients, could be studied further. Three of

the TLL clones responded specifically to *M. leprae,* while one of the clones exhibited a broad crossreactivity to other mycobacteria. All of these clones were of the CD4+CD8− phenotype. Our findings suggest that responsiveness to *M. leprae* can be detected *in vitro* in a proportion of LL patients who have undergone prolonged chemotherapy, and that this response involves *M. leprae*-reactive CD4+CD8− T cells, of which some appear to be specific to *M. leprae.*—Authors' Summary

González-Abreu Castells, E., González Segredo, A., Mariño Albernas, J. and Verez Bencomo, V. [Serologic demonstration of the activity of a *Mycobacterium leprae* antigen stained by chemical synthesis.] Rev. Cub. Med. Trop. **41** (1989) 10–17. (in Spanish)

Following the chemical isolation and characterization of the phenolic glycolipid-I by Hunter and Brennan in 1981, derived from infected armadillo liver, studies were continued to achieve the chemical synthesis of this trisaccharide, which is part of the glycolipid and, as has been demonstrated, was the major antigenic determinant of this substance. The synthetic antigen obtained by Fujiwara in 1984 and Gigg in 1985 was conjugated with bovine albumin. Immunodominance of the terminal residue 3, 6-di-O-methyl-glucose was confirmed by the use of ELISA, monoclonal and polyclonal antibodies. In Cuba, based on this knowledge, Mariño and Verez obtained the antigen by another way of synthesis conjugated with acrylamide against positive and negative (71%) control sera, as well as its specificity in the reaction with sera from tuberculous patients and children vaccinated with BCG (89%).—Authors' English Summary

Happ, M. P., Kubo, R. T., Palmer, E., Born, W. K. and O'Brien, R. L. Limited receptor repertoire in a mycobacteria-reactive subset of γδ T lymphocytes. (Letter) Nature **342** (1989) 696–698.

The physiological role of lymphocytes bearing the γδ T-cell receptor (TCR) is still unclear. A function for a subset of these cells, however, is inferred from the finding that certain γδ chain-bearing lymphocytes are stimulated in a receptor-dependent fashion by mycobacterial antigens. We found that hybridomas derived from such cells in newborn murine thymus not only responded to mycobacterial purified protein derivative (PPD), but also exhibited an apparent autoreactivity. In neither response was haplotype-specific major histocompatibility (MHC) restriction demonstrable. To investigate the nature of antigen recognition by these γδ+ cells, we sequenced the γ- and δ-chains from 28 PPD-reactive hybridomas, and found that a specific γ-chain, together with one of a limited set of δ-chains, was needed to generate the PPD specificity. The reactive γδ pairs exhibited considerable junctional diversity, which may act to produce differences in the fine specificities of the responding cells.—Authors' Abstract

Izumi, S., Fujiwara, T., Ikeda, M., Nishimura, Y., Sugiyama, K. and Kawatsu, K. Novel gelatin particle agglutination test for serodiagnosis of leprosy in the field. J. Clin. Microbiol. **28** (1990) 525–529.

We developed a novel gelatin particle agglutination test (MLPA) for the serodiagnosis of leprosy; this test is especially useful for clinical practice and epidemiological surveys of leprosy in countries in which the disease is endemic. The antigen used in the test is the chemically synthesized trisaccharide moiety of *Mycobacterium leprae*-specific phenolic glycolipid-I. MLPA is a simple and easy technique having sensitivity and specificity comparable to those of the conventional indirect enzyme-linked immunosorbent assay. The new technique was found to be useful for monitoring of chemotherapy and predictive diagnosis of high-risk individuals in contact with persons with leprosy and may be useful for the prediction of relapse. We are now preparing to supply a quality-controlled ready-to-use MLPA kit for leprosy control in countries in which leprosy is endemic.—Authors' Abstract

Jayapal, N., Shanmugasundaram, N., Thomas, P. A., Valli, P. R. T., Thyagarajan, S. P. and Subramanian, S. A simple method to quantitate circulating immune complexes in different diseases. Indian J. Pathol. Microbiol. **32** (1989) 33–39.

Immune complexes are involved in the pathogenesis of many diseases of varied etiology such as autoimmune disorders, protozoal diseases, bacterial and viral infections. Quantitation of immune complexes in these diseases can be used for diagnosis and to ascertain the prognosis. The simple method of precipitation by polyethylene glycol and quantitation by single radial immunodiffusion has been used in leprosy, syphilis, bacterial endocarditis and systemic lupus erythematosus (SLE). This method found significantly higher levels of circulating immune complexes (CICs) in erythema nodosum leprosum, culture-positive bacterial endocarditis and SLE where CICs are known to play an important role in the pathogenesis.—Authors' Abstract

Kapur, J., Moudgil, K. D., Chopra, S. P. and Gupta, S. K. Evaluation of enzyme immunoassays using purified protein derivative (PPD) and its pool fractions 3 and 4 for diagnosis of pulmonary tuberculosis. Indian J. Chest Dis. All. Sci. **31** (1989) 25–32.

A critical evaluation of two enzyme immunoassays (EIAs) for the diagnosis of pulmonary tuberculosis is reported. Purified protein derivative (PPD) or its pooled fractions 3 and 4 were used as antigens for detection of antibodies in sera from 53 patients with active pulmonary tuberculosis and 10 normal healthy individuals. The cut-off point for each EIA was based on the absorbance (mean + 3 S.D.) of normal sera with the respective antigens. All of the normal sera were negative in both assays. The positivity of tuberculosis patients in either assay was 86.8%. Thus, for serodiagnosis of tuberculosis fractions 3 and 4 of PPD could serve as a good substitute for whole PPD. Sera from 45 leprosy patients were also analyzed to assess the specificity of the EIAs. The mean reactivity of tuberculoid leprosy sera was comparable to that of normal sera. The ratio of the mean absorbance of lepromatous leprosy (LL) sera and normal sera was 16.73 with PPD, in comparison to 21.95 for pooled fractions 3 and 4. Out of 10 LL patients, 9 (90%) were positive with fractions 3 and 4, in comparison to 10 (100%) with PPD; 71.1% of leprosy patients belonging to different categories were positive in the assay based on PPD in comparison to 64.4% in EIA using fractions 3 and 4. The high false-positivity of leprosy sera in an assay designed for detection of pulmonary tuberculosis has immense implications in interpretation of results of the assay for diagnostic and epidemiological purposes.—Authors' Abstract

Kaufmann, S. H. E., Flesch, I. E. A., Munk, M. E., Wand-Württenberger, A., Schoel, B. and Koga, T. Cell-mediated immunity to mycobacteria: a double-sided sword? Rheumatol. Int. **9** (1989) 181–186.

Mycobacteria are intracellular pathogens capable of replicating in resting macrophages. Specific helper-T lymphocytes which activate antimycobacterial capacities in infected macrophages represent an important constituent of acquired resistance. In addition, cytolytic-T lymphocytes may contribute to resistance. On the other hand, lysis of infected host cells may also comprise autoaggressive consequences. Recent evidence suggests that T cells with specificity for mycobacterial heat-shock proteins are involved in the antimycobacterial immune response. Heat-shock proteins are evolutionarily highly conserved and crossreactivity between microbial and mammalian molecules may occur on the B-cell and T-cell level. Thus, T cells directed against shared epitopes of mycobacterial and autologous origin could initiate autoimmune reactions.—Authors' Summary

Kehrer, D., Narayanan, R. B., Malaviya, G. N. and Girdhar, B. K. Isolation and characterization of cells in granulomas of nerves of leprosy patients. Acta Leprol. (Genève) **7** (1989) 7–11.

Single cell suspension from the granulomas in nerves of leprosy patients were prepared for an *in vitro* study of the properties of infiltrating cells. Nerve biopsies from 17 patients with tuberculoid (N = 9) and lepromatous (N = 8) leprosy cases were analyzed. The granulomas were found to contain lymphocytes and macrophages. Lymphocytes were the predominant infiltrating cells in the tuberculoid nerves. In contrast, lepromatous nerves contained very few of these cells. The majority of lympho-

cytes in tuberculoid granulomas were activated T cells because they formed rosettes with sheep erythrocytes, exhibited esterase dots in the cytoplasm, and expressed HLA-DR antigens. A small proportion of the lymphocytes also formed rosettes with EAC. Most macrophages from both the granulomas were mature macrophages because they were esterase positive, did not exhibit peroxidase activity, and expressed HLA-DR antigens. The macrophages did not possess C3 surface receptors.—Authors' Summary

Kumar, V., Narayanan, R. B. and Girdhar, B. K. Comparison of the characteristics of infiltrates in skin and nerve granulomas of leprosy. Acta Leprol. (Genève) **7** (1989) 19–24.

The characteristics of infiltrates in the dermal and neural granulomas from the same leprosy patients were compared by preparing a single cell suspension. Skin and nerve biopsies from 10 patients, 5 with tuberculoid and 5 with lepromatous leprosy, were analyzed. The granulomas contained lymphocytes and macrophages. Lymphocytes were the predominant infiltrating cells in the tuberculoid dermal and neural granulomas. A high proportion of lymphocytes in both the skin and nerve granulomas in these cases were activated T cells because they formed rosettes with sheep erythrocytes and expressed HLA-DR antigens. In contrast, lepromatous dermal and neural granulomas contained very few of these lymphocytes. Dermal and neural granulomas from both of the types of leprosy contained mature macrophages because they were esterase positive, did not exhibit peroxidase activity, and expressed HLA-DR antigens. These macrophages did not possess C3 surface receptors either. These findings suggest that the infiltrates in the skin and nerve granulomas of a given type of leprosy have similar characteristics.—Authors' Summary

Launois, P., Maillere, B., Dieye, A., Sarthou, J. L. and Bach, M.-A. Human phagocyte oxidative burst activation by BCG, *M. leprae,* and atypical mycobacteria: defective activation by *M. leprae* is not reversed by interferon γ. Cell. Immunol. **124** (1989) 168–174.

The activation of the phagocyte oxidative respiratory burst by various mycobacteria was evaluated in an *in vitro* assay, by measuring the chemiluminescence, associated to the release of oxidizing species, generated by normal human whole blood phagocytes. All mycobacterium species, except *Mycobacterium leprae,* induced a significant chemiluminescence response. The strongest stimulus was provided by BCG, followed by *M. triviale, M. chelonei,* and *M. fortuitum. M. kansasii, M. intracellulare,* and *M. lepraemurium* elicited a weak response, although higher than that triggered by *M. leprae.* Both polymorphonuclear and mononuclear cells contributed to the whole blood cell chemiluminescence stimulated by mycobacteria, mononuclear cells being more efficient on a per cell basis. Phagocyte activation by recombinant interferon-γ did not improve *M. leprae* ability to trigger a significant chemiluminescence response. The failure of *M. leprae* and of some atypical mycobacteria to stimulate a strong phagocyte oxidative respiratory burst may have some relevance to their pathogenicity.—Authors' Summary

Mackworth-Young, C. G. Cross-reactive idiotypes in sera from patients with leprosy, lupus and Lyme disease and from healthy individuals. Clin. Exp. Immunol. **79** (1990) 78–82.

Monoclonal IgM autoantibodies have previously been generated from a patient with lepromatous leprosy. Polyclonal anti-idiotypes raised against two of these monoclonal antibodies (8E7 and TH9) were used in an immunoassay to detect the presence of idiotype in human serum. The anti-idiotypes recognize different but overlapping sets of idiotypic determinants, some of which are present on antibodies which bind to *Mycobacterium leprae.* Sera were tested from 16 individuals with leprosy, 45 with systemic lupus erythematosus, 20 with Lyme disease, and 80 healthy subjects. Positive sera were detected in all groups (seven, two, three, and four, respectively). In most cases the serum bound to both anti-idiotypes, the idiotype being present in the IgM and/or IgG fraction. Levels of the two idiotypes varied independently of total serum IgG concentration and, in serial serum samples

from one patient, independently of each other. The results indicate that 8E7 and TH9 may be representative of serum antibodies which are commonly expressed in leprosy, but may also be expressed in other diseases and in health; and they suggest that such serum antibodies are encoded by a widely shared set of variable region genes.—Authors' Summary

Marolia, J., Robinson, P. and Mahadevan, P. R. A complex component modulating immune-deficient cells in leprosy patients leading to loss of viability of *Mycobacterium leprae*—a possible vaccine. Clin. Exp. Immunol. **79** (1990) 7–14.

Macrophages from peripheral blood of leprosy patients, both multibacillary and paucibacillary are unable to kill phagocytosed *Mycobacterium leprae* due to their inability to produce superoxide (O_2^-) and hydroxyl radicals (OH·). The macrophages from healthy individuals are able to kill *M. leprae* along with release of O_2^- and OH· radicals. The deficiency in the macrophages of both types of leprosy patients is removed by activation of these cells when exposed to a culture supernatant obtained after stimulation of peripheral blood mononuclear cells from the same patients with delipidified cell components of *M. leprae* which are most likely cell-wall proteins. The activation of macrophages also leads to recognition of whole live *M. leprae* as an antigen by cells from lepromatous patients. This activation of the phagocytes by delipidified cell components is blocked by cyclosporin A, indicating the possible role of several steps involved in immune activation of cells. The observations thus indicate the significant ability of delipidified cell components to eliminate the deficiencies in the macrophages from leprosy patients and restore them to behave like the cells from healthy individuals. Considering all these, it is suggested that delipidified cell components could be potential modulators, and are probably capable of functioning as a vaccine for leprosy.—Authors' Summary

Mohagheghpour, N., Munn, M. W., Gelber, R. H. and Engleman, E. G. Identification of an immunostimulating protein from *Mycobacterium leprae*. Infect. Immun. **58** (1990) 703–710.

Despite the recent identification of a number of *Mycobacterium leprae* proteins, the major immunogenic determinants of this organism remain obscure. We isolated from *M. leprae* a potent immunostimulatory preparation, designated the MLP fraction, which contains a major protein of 35 kilodaltons (kDa). This protein was precipitated by monoclonal antibody ML03-A_1, which recognizes a 35-kDa protein of *M. leprae,* and by sera obtained from patients with lepromatous leprosy. Neither sera from healthy controls nor sera from patients with pulmonary tuberculosis recognized the 35-kDa protein, and only 1 of 4 serum samples from patients with borderline tuberculoid leprosy reacted with this protein. The MLP fraction stimulated T-cell proliferation in patients with leprosy whose T cells proliferate in response to whole *M. leprae* cells. Apparently, the T-cell epitope associated with MLP is also expressed on *M. tuberculosis* and *M. bovis* BCG, since patients with pulmonary tuberculosis and BCG-vaccinated individuals demonstrated significant responses to the MLP fraction. The 35-kDa *M. leprae* protein, purified to homogeneity in the laboratory of P. J. Brennan, stimulated T-cell proliferative responses in all MLP-responsive subjects. These findings suggest that the 35-kDa protein present in MLP is an immunostimulatory component of *M. leprae.* In addition to serving as a useful probe for study of the T-cell anergy associated with lepromatous disease, this protein may ultimately be useful as a component of a vaccine designed to provide protection against infection with *M. leprae.*—Authors' Abstract

Molloy, A., Gaudernack, G., Levis, W. R., Cohn, Z. A. and Kaplan, G. Suppression of T-cell proliferation by *Mycobacterium leprae* and its products: the role of lipopolysaccharide. Proc. Natl. Acad. Sci. U.S.A. **87** (1990) 973–977.

Addition of soluble molecules obtained from sonicated *Mycobacterium leprae* markedly suppressed the proliferative response to the mitogen anti-CD3 of peripheral blood mononuclear cells and isolated

T cells. Suppression was nonspecific and occurred with cells from lepromatous and tuberculoid leprosy patients as well as control donors. The purified lipoarabinomannans from *M. leprae* and *M. tuberculosis* had a similar spectrum of inhibition; whereas their deacylated derivatives were without effect. All mycobacterial preparations of either a crude or purified state, which suppressed cellular responses, contained appreciable quantities of bacterial lipopolysaccharide by the *Limulus* amebocyte assay. Contamination with lipopolysaccharide could account for the extent and nonselectivity of the T-cell suppression. Suppression was also monocyte-dependent and in part due to the release of arachidonate metabolites of the cyclooxygenase pathway.—Authors' Abstract

Moudgil, K. D., Gupta, S. K. and Talwar, G. P. Generation and characterization of a human monoclonal antibody against phenolic glycolipid-I of *M. leprae.* Indian J. Lepr. **61** (1989) 479–484.

The development of an Epstein-Barr virus transformed human B-cell line secreting a monoclonal antibody (MoAb), KR2/B5, is described. KR2/B5 is an IgM type of antibody and is highly specific for phenolic glycolipid-I (PGL-I) a component unique to *Mycobacterium leprae.* The MoAb appears to be directed against the terminal sugar residue of the immunodominant trisaccharide component of PGL-I.—Authors' Abstract

Narayanan, R. B. and Girdhar, B. K. *In vitro* studies on dermal leprosy granulomas: assessment of division and protein synthesis of cells. Acta Leprol. (Genève) **7** (1989) 13–17.

Single cell suspension from dermal leprosy granulomas (10 tuberculoid and 10 lepromatous) was prepared, and an assessment of the division and protein synthesis by the cells was made. The cells of tuberculoid granulomas showed a high incorporation of ^{3}H-thymidine and ^{14}C-leucine. On the contrary, the cells of the lepromatous granulomas exhibited poor division but their protein synthesis remained unimpaired. These observations suggest that the epithelioid cell granuloma of tuberculoid leprosy appears to be more active and secretory than the macrophage granuloma of lepromatous leprosy.—Authors' Summary

Saint-André, P., Baquillon, G. and David, M. [Immunomodulating therapy of multibacillary leprosy using extracts of bacterial ribosomes.] Acta Leprol. (Genève) **7** (1989) 57–58. (in French)

Ribomunyl® can in practice be profitably included in the range of immunostimulants, but it requires weekly injections and hence surveillance that will also enable any adverse reactions to be monitored. Its action and tolerance after polychemotherapy have still to be studied. On the basis of our experience, we feel that only isoprinosine, whose effectiveness and good tolerance we have demonstrated, can be used for self-treatment in a mass campaign.—Authors' English Summary

Sasiain, M. del C. and de la Barrera, S. [Immunosuppression in leprosy.] Medicina (Buenos Aires) **49** (1989) 213–215. (in Spanish)

Many authors tried to show that in lepromatous leprosy (LL), suppressor mechanisms are involved in the immune response. We have previously shown that nonspecific suppression (ConA induced) was impaired in LL patients and tends to normalize during the erythema nodosum leprosum (ENL) episode. In this system we have shown that CD8+ cells (Leu2a^{+}) can interfere with the generation of ConA-induced suppression. We also observed that a high percentage of LL patients had an increased spontaneous suppression. In these patients, the number of Leu2a^{+} cells added in the assay did not correlate with the suppression values. On the other hand, we had demonstrated that the monocyte suppressor system may have an important role, due to the release of soluble factors (PGE2). We evaluated *Mycobacterium leprae*-induced suppressor-cell function using a two-step assay on T-cell proliferation. The results of this study indicate that the ability of *M. leprae* to induce suppressor activity was lower in LL patients than in tuberculoid (TT), intermediate clinical forms (BB, BL, BT) and BCG-immunized controls. On the other hand, we de-

termined that the proportion of peripheral blood mononuclear cells (PBMC) bearing the Leu8$^+$ antigen (associated with suppressor inducer cells) was low in LL and tends to normalize during the ENL episode. Suppression of proliferation could not be overcome with exogenous IL-2 and was not related to the induction of Tac antigen. The ability of LL, TT, ENL and normal cells to proliferate upon PHA or ConA stimulus was similar. These results indicate that the defect in the generation of *in vitro* suppression by *M. leprae* in LL occurred during the induction period and could be associated with the reduction of Leu8$^+$ cells. We believe that the low frequency of Leu8$^+$ cells in LL is a clue to the inability of LL-PBMC to generate specific and nonspecific suppression of T-cell proliferation.—Authors' English Summary

Suárez Moreno, D., Valdés Portela, A., González Abreu, E. and Aparicio Gómez, J. [Circulating immune complexes in multibacillary leprosy patients and their relationship with anti-*M. leprae* antibodies and the major immunoglobulin classes.] Rev. Leprol. Fontilles **17** (1989) 259–265. (in Spanish)

A study of the immune humoral response was made in patients of multibacillary leprosy, those living with the patients and healthy individuals without known contact with leprosy. They were divided into three groups: patients with less than 2 years of treatment, patients with more than 2 years of treatment with satisfactory evolution, and patients with more than 2 years of treatment presenting erythema nodosum leprosum episodes. The circulating immune complex levels were determined by the polyethylene glycol 6000 precipitation method, the antibodies levels of anti-*Mycobacterium leprae* by ELISA technique employing the analogous semisynthetic antigen of phenolic glycolipid-I of *M. leprae* and the immunoglobulins IgA, IgG and IgM were quantified by radial immunodiffusion. In patients with type 2 reaction, the immune complex values and IgG and IgA immunoglobulins were higher than in the rest of the groups. The IgM and the anti-*M. leprae* antibodies decreased during the treatment time, both tending to have an inverse relation with the immune complexes.—Authors' English Summary

van Schooten, W. C. A., Elferink, D. G., van Embden, J., Anderson, D. C. and de Vries, R. R. P. DR3-restricted T cells from different HLA-DR3-positive individuals recognize the same peptide (amino acids 2-12) of the mycobacterial 65-kDa heat-shock protein. Eur. J. Immunol. **19** (1989) 2075–2079.

Studies in experimental animals have demonstrated that the T-cell response to immunogenic proteins is limited to one or a few epitopes on such proteins and that the MHC haplotype of the responder is an important factor in determining which epitope is recognized (immune response gene effect). However, if and to what extent MHC genes control the immune response to pathogens in man is virtually unknown. We have studied the human T-cell response to the mycobacterial 65-kDa heat-shock protein, a major immunogen of *Mycobacterium leprae* and *M. tuberculosis,* the causative agents of leprosy and tuberculosis, respectively, in relation to HLA-DR phenotype. In a large panel of short-term cultured polyclonal anti-mycobacterial T-cell lines, from 45 different individuals representing all DR-restriction specificities, only DR1- and DR3-restricted T-cell lines proliferated to the 65-kDa protein. The DR1-restricted T-cell lines responded to three new epitopes on the mycobacterial 65-kDa protein, one of which is specific for the *M. tuberculosis* complex. Altogether nine T-cell epitope-containing regions have now been mapped on the 65-kDa protein, and the response to each of them was exclusively restricted via one HLA-DR allele. Most importantly, all six 65-kDa-responsive DR3-restricted T-cell lines from different individuals recognized an epitope on the same peptide, representing amino acids 2–12 of the 65-kDa protein, that was previously mapped using DR3-restricted T-cell clones. From these data we conclude that the human T-cell response to both the whole mycobacterial 65-kDa heat-shock protein and to defined epitopes on this protein is controlled by HLA-DR genes. The mycobacterial 65-kDa protein has been

implicated in the design of subunit vaccines against tuberculosis and leprosy as well as the induction of immunopathology. In both instances the Ir gene control of the T-cell response to this protein may have to be taken into account.—Authors' Abstract

Wang, C.-R., Liu, M.-F., Hsieh, R.-P., Chuang, C.-Y. and Cheng, C.-S. Evaluation of humoral immunity on leprosy patients in Taiwan: a preliminary report. J. Formosan Med. Assoc. **88** (1989) 669–672.

Twenty-four tuberculoid (T)-type and 31 lepromatous (L)-type leprosy patients from Taiwan Provincial Lo-Sheng Leprosarium were enrolled in this study. Twenty-six age- and sex-matched normal subjects were also studied as a control group. The evaluation of their general and specific humoral immunity included B-cell subpopulations, three major classes of immunoglobulin (G, A and M) and antibodies in the IgG class against lepromin suspension and bacillus Calmette-Guérin (BCG) sonicate. T-type patients showed a larger B-cell percentage than L-type patients ($p < 0.01$). In general, patients with leprosy, both T and L types, had higher serum immunoglobulin levels than the control group. T-type patients showed greater antibody levels than the control group ($p < 0.05$ for anti-lepromin and $p < 0.0001$ for anti-BCG). L-type patients demonstrated a higher anti-BCG IgG level than the control group ($p < 0.0001$). The level of anti-BCG IgG was more frequently above the cutoff level than that of anti-lepromin IgG in leprosy patients ($p < 0.01$ for T, $p < 0.005$ for L). In conclusion, humoral immunity is not impaired in leprosy patients. Discrepancies for T- and L-type patients among B-cell subpopulation, serum immunogobulin levels and specific antibody levels reflect different aspects of cell-mediated immunity impairment. Although leprosy patients had elevated anti-BCG IgG levels, it is impossible to differentiate L- and T-type patients; specific antigens are needed for serodiagnosis of leprosy patients in Taiwan.—Authors' Summary

Weng, X.-M., *et al.* [Evaluation of Ms-ELISA as a screening test for leprosy.] China Lepr. J. **5** (1989) 190–193. (in Chinese)

The levels of the antibodies in the blood of newly found leprosy patients, patients being treated and their household contacts, and healthy controls in endemic and non-endemic areas were examined with Ms- and PGI-ELISA. The results show that the levels of both Ms-IgG and PGI-IgM can equally display the correlation between the degree of infection and the load of the bacilli, but comparison with PGI-ELISA shows that specificity of Ms-ELISA is lower and Ms-ELISA may not be used in low-endemic areas alone. During the period of treatment, the level of Ms-IgG antibody will vary with the drug used and the duration of taking the drug treatment and, therefore, it may serve as an indication of the efficiency of treatment.—Authors' English Abstract

Wu, Q.-X., *et al.* [Ms-ELISA for detection of serum antibody level in leprosy patient—establishment of Ms-ELISA.] Chung Kuo I Hsueh Ko Hsueh Yuan Hsueh Pao **10** (1988) 451–455. (in Chinese)

In this article, we report the establishment of (Ms)-ELISA and its comparison with PGL-I-ELISA. Sera for the test were collected from leprosy patients (152 cases), tuberculosis patients (20 cases), and normal subjects (120 cases, in nonendemic area of leprosy). The results indicated that Ms-ELISA was provided with high sensitivity (99%), specificity (95%), and significant positive correlation with PGL-I-ELISA ($r = 0.6106$, $p < 0.0001$), the positive correlation rate between them being over 90%. In Ms-ELISA, results of nonparametric test indicated that there was highly significant correlation ($r_s = 0.955$, $p < 0.01$) between OD values and BI. These data suggest that Ms-ELISA is valid and reliable. Since Ms-ELISA is more simple and easy to get antigen and reagents, it may be useful in leprosy control programs, both for screening subclinical infection and follow up of drug response.—Authors' English Abstract

Wu, Q.-X., *et al.* [Study on monoclonal antibody in leprosy. I. Production of monoclonal antibody against antigenic epi-

tope of PGL-I specific to *M. leprae.*] China Lepr. J. **5** (1989) 184–188. (in Chinese)

The authors report that four hybridoma cell lines, which secrete monoclonal antibodies (McAb), have been produced by means of the fusion between mouse myeloma cells SP2/O and spleen cells from BALB/c mice immunized with whole *Mycobacterium leprae* plus the unique phenolic glycolipid-I (PGL-I) of *M. leprae.* Three out of them, which were named D_{10}, C_4, and G_3, produced McAbs against *M. smegmatis.* Only one of them (named H_2) secreted McAbs against PGL-I and *M. smegmatis,* and because H_2 lost the ability to secrete antibodies against *M. smegmatis* after several selecting passages, H_2* was named for it. The McAb secreted by H_2* showed high specificity to PGL-I, NT-O-BSA and ND-O-BSA, and no crossreaction to 13 strains of mycobacteria tested in a primary identification test with the exception of very weak crossreactivity to *M. tuberculosis* and *M. xenopi.*

H_2* could produce ascitis fluids with higher titer McAb after being inoculated into BALB/c mice i.p. The McAb secreted by the H_2* hybridoma cell line is a mixture with major IgG and minor IgM classes of mouse, and H_2* is stable to secrete McAb mentioned above in tissue culture after 66 subcultures over 6 months and the storage of over 11 months in liquid nitrogen. Some problems of the established cell line are briefly discussed.—Authors' English Abstract

Zhang, X.-Q., *et al.* [Deposition of immunoglobulin and complement in the skin of leprosy patients.] China Lepr. J. **5** (1989) 193–196. (in Chinese)

The authors examined the deposition of immunoglobulin and complement in the skin of 74 patients with all forms of leprosy, using immunofluorescent and immunoelectroscopic techniques, and found that the deposit at the dermo-epidermal junction, around the appendages and in the blood vessels of the skin is an immune complex, and its quantity is parallel with the load of *Mycobacterium leprae.* The authors believe that the immune complex comes from the circulating blood and can activate complement, thus producing inflammation.—Authors' English Abstract

Microbiology

Besra, G. S., Minnikin, D. E., Sharif, A. and Stanford, J. L. Characteristic new members of the phthiocerol and phenolphthiocerol families from *Mycobacterium ulcerans.* FEMS Microbiol. Lett. **66** (1990) 11–13.

Diacyl phthiodiolone A and phenolphthiodiolone A lipids were isolated from two strains of *Mycobacterium ulcerans.* The diol units of the phthiodiolone A and phenolphthiodiolone A components were shown to have *erythro* stereochemistry by infrared spectroscopy and proton nuclear magnetic resonance of an acetal derivative. This stereochemistry is shared only by related diols from *M. marinum,* the diols from *M. bovis, M. kansasii, M. leprae* and *M. tuberculosis* having *threo* stereochemistry.—Authors' Summary

Bhatia, V. N. Effect of temperature, cholesterol and nerve tissue on multiplication of armadillo *M. leprae.* Indian J. Lepr. **61** (1989) 453–457.

The effect of temperature, nerve tissue and certain constituents of the medium on multiplication of armadillo *Mycobacterium leprae* was studied using Hanks' balanced salt solution (HBSS). An equal or better growth was seen at 30°C and 10°C compared to 37°C. Multiplication was also seen at −20°C. Adding cholesterol, fetal calf serum, cystine-HCl, sodium thioglycolate or nerve suspension and covering medium with liquid paraffin each showed beneficial effect. HBSS containing fetal calf serum, cholesterol with sodium thioglycolate or cystine-hydrochloride showed maximum multiplication. These combinations may be used for

testing additional factors for further improvement of the medium.—Author's Abstract

Chatterjee, B. R. and Roy, R. D. Growth of *Mycobacterium leprae* in a redox system: III. Evidence of growth at low temperature (psychrophilia) and further refinement of growth medium. Indian J. Lepr. **61** (1989) 458–466.

Considerable growth enhancement, largely as non-acid-fast, slender and long rods has been seen when incubated at 10°C. Concentration of some of the media constituents have been reduced that has improved the quantum of growth. A remarkable proneness to physical disintegration of the grown bacilli has been seen and its significance discussed. Also, the possible immunogenic advantage of non-acid-fast *Mycobacterium leprae* has been discussed. The question of identification is still not solved, and work is in progress.—Authors' Abstract

Jackson, M., Chan, R., Matoba, A. Y. and Robin, J. B. The use of fluorescein-conjugated lectins for visualizing atypical mycobacteria. Arch. Ophthalmol. **107** (1989) 1206–1209.

We investigated the feasibility of using fluorescein-conjugated lectins for visualizing and differentiating two species of atypical mycobacteria. Pure cultures of *Mycobacterium fortuitum* and *M. chelonei* were established, as was an experimental model of infectious keratitis involving these two organisms. Samples from the pure cultures and corneal scrapings were placed on glass slides, fixed, and incubated with one of a panel of 22 fluorescein-conjugated lectins. The slides were examined using an epifluorescence microscope. Fluorescein-conjugated concanavalin A brightly stained both species of atypical mycobacteria, in both the pure culture and experimental keratitis samples. Several additional fluorescein-conjugated lectins (wheat germ agglutinin, succinylated wheat germ agglutinin, *Phaseolus vulgaris* erythroagglutinin, and *Psophocarpus tetragonolobus* agglutinin) brightly stained *M. chelonei,* but only moderately stained *M. fortuitum.* These staining patterns are consistent with the known carbohydrate compositions of the cell walls of atypical mycobacteria and suggest that fluorescein-conjugated lectins may be useful for the visualization of these organisms in corneal infections.—Authors' Abstract

Miranda, R. N., Sounis, E. L. M., Emmel, T., Dechandt, H. S., Miranda, R. P. G. and Dechandt, I. T. [Culture of mycobacteria in human plasma.] An. Bras. Dermatol. **64** (1989) 291–296. (in Portuguese)

In view of the claimed uncultivability of *Mycobacterium leprae* in artificial laboratory media, one of the authors (RNM) imagined the use of human plasma from healthy individuals as an original medium. After 60 experiments done since the year 1980 to date, an evaluation of the results obtained allows the disclosure of the following data: 1) Over 50% of the experiments performed denoted bacterial growth in the culture tubes. 2) Other culture media have not shown similar growth. 3) The bacterial morphology—rods and globi—was similar to *M. leprae.* 4) Staining techniques revealed that the bacteria were alcohol-acid-fast and gram-positive. 5) The pyridine test inhibited acid-fastness in the bacteria as occurs with *M. leprae.* 6) The dopa-test was favorable to *M. leprae.* 7) Inoculated guinea pigs did not present tuberculosis and any other disease. 8) A bacillary lepromin prepared with the cultures produced positive and negative reactions, respectively, in T-form and L-form patients of leprosy. 9) The authors would like other researchers to repeat the experiment and confirm these results.—Authors' English Summary

Papa, F., Cruaud, P. and David, H. L. Antigenicity and specificity of selected glycolipid fractions from *Mycobacterium tuberculosis.* Res. Microbiol. **140** (1989) 569–578.

Antigenicity of *Mycobacterium tuberculosis* glycolipids polyphthienoyl trehalose (PPTR), phenolic glycolipid (PGL-Tb1), tetraacyl trehalose-2′-sulfate (SL-I) and diacyl trehalose-2′-sulfate (SL-IV) was examined in rabbits. PPTR did not induce production of IgG antibodies in rabbits, while PGL-Tb1, SL-I and SL-IV glycolipids were effi-

cient in this respect. Immune sera raised in rabbits immunoreacted exclusively with the corresponding antigens, which indicated that they were remarkably specific. Specificity of the immune sera was further examined using crude extracts of representative strains of 39 mycobacterial species, and the data showed that these immune sera reacted only with extracts of *M. tuberculosis* and *M. africanum*. An antiserum raised against whole cells of *M. leprae* immunoreacted with the purified SL-IV antigen from tubercle bacilli.—Authors' Summary

Sritharan, V., Wheeler, P. R. and Ratledge, C. Aspartate metabolism in *Mycobacterium avium* grown in host tissue and axenically and in *Mycobacterium leprae*. J. Gen. Microbiol. **136** (1990) 203–209.

Aspartokinase activity was detected in extracts from *Mycobacterium leprae* (recovered from armadillo liver) and in *M. avium* grown axenically and *in vivo*. Homoserine dehydrogenase activity was only detected in *M. leprae* and in *M. avium* grown axenically. Activities, when detected, were 50% to 70% lower in *M. leprae* or *M. avium* grown *in vivo* than in axenically grown *M. avium*. In these two pathogenic mycobacteria, aspartokinase and homoserine dehydrogenase are subject to feedback inhibition by methionine—an additional regulator over those observed for the enzymes from *M. smegmatis*. Intact mycobacterium incorporated carbon from [U-^{14}C]aspartate into the aspartate family of amino acids (threonine, isoleucine, methionine and lysine) though the rate of incorporation in *M. avium* grown *in vivo* was about half that in *M. avium* grown axenically.—Authors' Abstract

Wheeler, P. R. Biosynthesis and scavenging of pyrimidines by pathogenic mycobacteria. J. Gen. Microbiol. **136** (1990) 189–201.

Mycobacterium microti incorporated a wide range of exogenously supplied pyrimidines into its nucleic acids. *M. avium* incorporated a relatively narrow range of pyrimidines but both *M. avium* and *M. microti* when recovered after growth *in vivo* incorporated a slightly wider range of pyrimidines than the same strains grown *in vitro*. *M. microti* and *M. leprae* could not take up uridine nucleotides directly but could utilize the pyrimidines by hydrolyzing them to uridine and then taking up the uridine. Pyrimidine biosynthesis, judged by the ability to incorporate carbon from CO_2 or aspartate into pyrimidines was readily detected in nongrowing suspensions of *M. microti* and *M. avium* harvested from Dubos medium, which does not contain pyrimidines. The biosynthetic activity was diminished in mycobacteria grown *in vivo* when there is likely to be a source of pyrimidines which they might use. Relative activities for pyrimidine biosynthesis *de novo* in *M. microti* were 100 for cells isolated from Dubos medium, 6 for cells isolated from Dubos medium containing the pyrimidine cytidine, and 11 from cells recovered after growth in mice. In contrast, relative activities for a scavening reaction, uracil incorporation, were 100, 71, and 59, respectively. Three key enzymes in the pathway of pyrimidine biosynthesis *de novo* were detected in *M. microti* and *M. avium*. Two, dihydroorotate synthase and orotate phosphoribosyltransferase, appeared to be constitutive in *M. microti* and *M. avium*. Aspartate transcarbamoylase activity was higher in these mycobacteria grown *in vivo* than in Dubos medium but it was repressed in *M. microti* or *M. avium* grown in Dubos medium in the presence of 50 μM pyrimidine. Aspartate transcarbamoylase was strongly inhibited by the feedback inhibitors ATP, CTP and UTP. Enzymes for scavenging pyrimidines were detected at low specific activities in all mycobacteria studied. Activities of phosphoribosyltransferases, enzymes that convert bases directly to nucleotides, were not related to the ability of intact mycobacteria to take up pyrimidine bases while activities of pyrimidine nucleoside kinases were generally related to the ability of intact mycobacteria to take up nucleosides. Phosphoribosyltransferase activity for uracil, cytosine, orotic acid and—in organisms grown in Dubos medium with 50 μM uridine—thymine, as well as kinases for uridine, deoxyuridine, cytidine and thymidine were detected in *M. microti*. However, *M. avium* only contained uracil and orotate phosphoribosyltransferase, uridine, cytidine and thymidine kinase,

and additionally deoxyuridine kinase when grown axenically with 50 μM uracil, reflecting its more limited abilities in pyrimidine scavenging.—Author's Abstract

Wheeler, P. R., Bulmer, K. and Ratledge, C. Enzymes for biosynthesis *de novo* and elongation of fatty acids in mycobacteria grown in host cells: is *Mycobacterium leprae* competent in fatty acid biosynthesis? J. Gen. Microbiol. **136** (1990) 211–217.

Fatty acid synthetase activity in extracts of *Mycobacterium leprae* was equivalent to 1:7 pmol malonyl-CoA incorporated into fatty acid min^{-1} (mg protein)$^{-1}$. This activity—if representative of living *M. leprae* organisms—is insufficient to enable them to synthesize their lipid requirements rapidly enough to support growth. The major activity for scavening fatty acids in extracts of *M. microti* and *M. avium,* as well as in extracts of *M. leprae,* was acetyl-CoA-dependent fatty acyl-CoA "elongase." This activity was about four times higher in *M. avium* and *M. microti* grown in a medium which contained lipids, or when grown in mice, than in medium without added lipids. In contrast, the *de novo* fatty acid synthetase activity was repressed in *M. avium* and *M. microti* when grown in medium that contained lipids, or when grown in mice. These results are consistent with the hypothesis that mycobacteria grown *in vivo* preferentially scavenge lipids from the host cells, and suggest that a source of lipid should be included in media for attempted axenic isolation of *M. leprae.*—Authors' Abstract

Woods, S. A. and Cole, S. T. A rapid method for the detection of potentially viable *Mycobacterium leprae* in human biopsies: a novel application of PCR. FEMS Microbiol. Lett. **65** (1989) 305–310.

A simple procedure based on the polymerase chain reaction has been developed to detect *Mycobacterium leprae,* rapidly and unambiguously, in biological samples. Its application to small numbers of *M. leprae* cells (~ 10^2) isolated from armadillo liver, mouse, foot pads or human biopsies is discussed.—Authors' Summary

Experimental Infections

Mitchell, I. C. and Turk, J. L. An experimental animal model of granulomatous bowel disease. Gut **30** (1989) 1371–1378.

A study has been undertaken of the granulomatous response induced in the ascending colon and terminal ileum of the guinea pig by the direct inoculation of mycobacterial antigens. Live BCG (Pasteur) 2×10^7 at 2 weeks induced epithelioid-cell granulomas in both the large and small bowel and in the draining lymph nodes. The area of infiltration was significantly greater for a given inoculum in the large bowel. Acid-fast bacilli (AFB) were present on Ziehl-Neelsen-stained sections of the large bowel infiltrate, but only rarely in sections from the small bowel lesions. The response to skin testing with a standardized amount of purified protein derivative (PPD) was less in animals inoculated in the small bowel. Inoculation with 2×10^9 cobalt-irradiated BCG gave rise, at 5 weeks, to granulomas containing lesser numbers of epithelioid cells and caseation was sometimes evident. There was a similar but smaller difference in the degree of infiltration at the two inoculation sites. Ziehl-Neelsen staining failed to reveal the presence of AFB in any sections of the bowel infiltrates. Skin testing with PPD gave a response which was greater in animals inoculated in the small bowel. An identical dose of cobalt-irradiated *Mycobacterium leprae* induced at 5 weeks a predominantly macrophage granuloma in both the large and small bowel, with no significant difference in the degree of infiltration at the two sites. No AFB were seen in Ziehl-Neelsen-stained sections of the bowel and skin testing with PPD was reduced. These findings and their

relevance to studies of the etiology of Crohn's disease are discussed.—Authors' Summary

Sanchez, A. and Foster, R. L. Effects of dietary composition on growth of *M. leprae* in mouse footpads. Indian J. Lepr. **61** (1989) 432–436.

The number of bacteria per mouse foot pad were measured at intervals beginning with the third month in male, weanling BALB/c mice infected with *Mycobacterium leprae* and fed for a period of 6 months to test the effects of diet on multiplication of bacteria. The mean bacteria count per foot pad in mice remaining at 6 months in the two high-fat diets was higher ($p = 0.014$) than the mean of the two low-fat diets. Likewise, the pooled mean bacterial count of mice fed the two diets of animal origin had a tendency to a higher mean bacterial count compared to mice fed the two diets of plant origin. Low levels of dietary protein in early life also seemed to predispose to *M. leprae* multiplication. Our data in mice suggest that the association of diet with human leprosy should be investigated.—Authors' Abstract

Truman, R. W., Job, C. K. and Hastings, R. C. Antibodies to the phenolic glycolipid-1 antigen for epidemiologic investigations of enzootic leprosy in armadillos (*Dasypus novemcinctus*). Lepr. Rev. **61** (1990) 19–24.

Other than man, nine-banded armadillos (*Dasypus novemcinctus*) are the only known natural hosts of leprosy with high rates of disease. The origin, range and risk of their infection is not yet clear and a better description of the rate of leprosy over the armadillo's range is needed. Both histopathological examination of armadillo ear tissues and serologic screening for IgM antibodies to the phenolic glycolipid-I (PGL-I) antigen of *Mycobacterium leprae* are good relative indices of enzootic prevalence. A survey of 216 armadillos from Louisiana and Florida, U.S.A., detected infection only among Louisiana animals. Average antibody prevalence (12.5%) was five times higher than the fully disseminated disease rate described histopathologically (2.7%). The differences in antibody and histopathological prevalence are due to the sensitivity of the methods for detecting early infection. Histopathological examinations describe an advanced disease. The higher antibody prevalence of wild armadillos is not likely to be the result of false-positive serologies from self-healing infections or other casual encounters with *M. leprae* as might be mimicked by lepromin injection. The environmental reservoir of *M. leprae* represented by infected armadillos is greater than could be previously estimated.—Authors' Summary

Wang, H.-Y., *et al.* [Study on inoculation of *M. leprae* to the tree shrew.] China Lepr. J. **5** (1989) 137–139. (in Chinese)

Tupaia belangeri yunalis (tree shrew) is a kind of primary primate animal. They were inoculated intravenously and subcutaneously into the foot pad with *Mycobacterium leprae* obtained from multibacillary leprosy patient. The foot pads of CFW mice were infected with the materials as controls. The results indicated that the foot pads of tupaia inoculated through the two routes showed evidence of the enhanced growth of acid-fast bacilli (AFB) 12 months postinoculation, while the numbers of AFB in the mice showed decline. Eighteen months after infection, the numbers of AFB in the tupaia foot pads increased up to 2.44×10^9 bacilli per gram of tissue. The foot pad of one tupaia showed a slight swelling and patchy granuloma was found in its histopathological sections. *M. leprae* were also seen in the branches of cutaneous nerve in the sections. Some macrophages revealed the tendency to be foamy, and a large number of AFB were found in a good many macrophages. The AFB appeared in some visceral tissues. The identification of the AFB in the tupaia is still being carried on.—Authors' English Abstract

Wang, H.-Y., Liu, J.-H., Ye, S.-Z., Yu, L.-C., Shi, M.-Q. and Sang, H.-G. Preliminary observations on experimental leprosy in tupaias (*Tupaia belangeri yunalis*). Lepr. Rev. **61** (1990) 12–18.

The *Tupaia belangeri yunalis* (tree shrew) is one of the primitive primates. They were inoculated subcutaneously in the foot pad or intravenously with *Mycobacterium lep-*

rae from a patient with multibacillary leprosy. As controls, the foot pads of CFW mice were inoculated with the same suspension of *M. leprae*. The results showed growth of acid-fast bacilli (AFB) in the foot pads of locally inoculated CFW mice and in the foot pads of both locally and intravenously inoculated tupaias. Whereas the numbers of AFB declined in the foot pads of CFW mice after 12 months, they increased in the tupaia foot pads, up to 2.44×10^9 AFB/g of tissue. The foot pads of one tupaia were swollen, which on section revealed a granulomatous infiltration, including foamy and heavily infected macrophages. *M. leprae* were also seen in the branches of cutaneous nerves. Also AFB occurred in some viscera. Preliminary studies indicate that the AFB multiplying in tupaias are *M. leprae*.—Authors' Summary

Winters, M. A. and Humphres, R. C. Induction of antigen-specific immunity and tolerance to *Mycobacterium leprae* in Lewis rats. Infect. Immun. **58** (1990) 495–501.

Intradermal (i.d.) immunization of Lewis rats with autoclaved *Mycobacterium leprae* resulted in antigen-specific proliferation responses and interleukin-2 (IL-2) release from spleen and lymph node cells that were detectable as early as 21 days, persisted for at least 9 months, and were dependent on the dose of antigen administered. Immunized animals were also completely resistant to a foot pad challenge with viable *M. leprae*. In contrast, intravenous (i.v.) administration of a least 10^8 irradiated *M. leprae* isolates induced a state of nonresponsiveness characterized by the absence of proliferation and IL-2 release by antigen-stimulated lymphoid cell cultures; however, *in vitro* responses to mitogenic stimulation and *in vivo* responses to keyhole limpet hemocyanin and *Listeria monocytogenes* were normal. Animals that received an i.v. injection of *M. leprae* remained nonresponsive to *M. leprae* antigens even after a subsequent i.d. immunization. This state of nonresponsiveness persisted for at least 6 months after induction. Results of foot pad challenge experiments showed that the ability of animals rendered nonresponsive by an i.v. injection of *M. leprae* to control the growth of viable *M. leprae* in the foot pad was not different from that of untreated rats. In addition, animals receiving an initial i.v. injection and a subsequent i.d. immunization with *M. leprae* were not protected from a viable challenge, as were rats that received only i.d. immunization. These results suggest that i.v. administration of a large dose of *M. leprae* to rats induces a state of nonresponsiveness to *M. leprae* antigens that may be similar to that seen in lepromatous leprosy patients.—Authors' Abstract

Epidemiology and Prevention

Albuquerque, M. de F. P. M., Morais, H. M. M. and Ximenes, R. [Increase in leprosy in the northeastern region of Brazil.] Rev. Saude Publica **23** (1989) 107–116.

The epidemiological aspects of leprosy in Recife from 1960 to 1985 were studied. Clinical-epidemiological records of 3923 leprosy patients reported to the Secretaria de Saúde do Estado de Pernambuco were reviewed. The [coefficient of detection] as well as the age, sex and type-specific detection rates were calculated. The way the cases were detected and the time elapsed between the appearance of the first symptoms and the disease were analyzed. The analysis of the time trend during the observation period showed an increase in the detection rate with time, rising from 5.5 per 100,000 inhabitants in 1960 to 36.1 per 100,000 inhabitants in 1985: The higher frequency of the tuberculoid type of leprosy and the high percentage of patients under 15 might reflect the expansion of the disease in Recife. The decline and the stabilization in the time elapsed between the appearance of the disease and its detection from 1979 onward, indicates a more prompt detection and, as a consequence of that, that the rate of detection is approaching the incidence rate.

From 1970 to 1985 the most common means of detecting leprosy cases was through dermatological consultation followed by disease notification. Only 14.2% of the cases were discovered through the surveillance of contacts. The analysis of the epidemiological and operational indicators suggests that the increase in the detection rate over the period from 1960 to 1985 was due both to expansion of the disease and improvement in control measures. The prevalence rate of leprosy in Recife in December 1984 was 2.04 per 100,000 inhabitants; according to the WHO criteria Recife may be considered an area of high endemicity.—AS/M. N. Lowenthal (Trop. Dis. Bull.)

Bechelli, L. M. Indeterminate leprosy in a population survey and in the subsequent follow-ups of children in Burma. Acta Leprol. (Genève) 7 (1989) 29–36.

The paper discusses various aspects of indeterminate (I) leprosy in the initial survey undertaken in the Burma BCG trial (69,242 inhabitants), and in the annual examinations of 28,220 children in the trial followed up over periods of 5 to 8 years. Age-specific rates in the initial mass survey are presented. In total 1914 cases were detected (6.2% I, 76% T, 16% L, and 1.8% B). Among the children in the BCG trial 768 cases were detected: 255 of them had the I form and their proportion (33%) was much higher than in the population survey. Of these 255 I cases only 4.3% had a negative or doubtful lepromin reaction. Two thirds of these 255 cases evolved to the tuberculoid pole in less than 1 year. No L cases appeared in the trial population until 10 and 11 years after the start of the trial. It is concluded that whereas a high proportion of indeterminate cases regress spontaneously or evolve toward the T pole, the indeterminate lepromin-negative cases are important in the dynamics of the disease because a proportion of them, if untreated, tend to envolve toward the L form. This stresses the importance of detection and treatment of I cases at any early stage in an effective strategy for controlling leprosy.—Author's Summary

Boudghene-Stambouli, O. and Merad-Boudia, A. [Reflections on leprosy in Algeria; a report on an autochtonous case in the Wilaya of Tlemcen (West Algeria).] Acta Leprol. (Genève) 7 (1989) 25–27. (in French)

Leprosy is not a problem for public health in Algeria. For one century (from 1888 to 1987), a maximum of 250 cases were reported, only 75 of them were Algerians and 61 caught the disease in Algeria. Hence, leprosy was mainly an imported disease. Will multiple exchanges with other countries increase the magnitude of the problem?—Authors' Summary

Chakrabarty, A. N. and Dastidar, S. G. Correlation between occurrence of leprosy and fossil fuels: role of fossil fuel bacteria in the origin and global epidemiology of leprosy. Indian J. Exp. Biol. 27 (1989) 483–496.

The authors have accumulated extensive data on the world distribution of fossil fuels (coal and oil) and propose the hypothesis that this distribution correlates with the present and historic distribution of leprosy. Such a correlation is supported by the authors' ability to isolate chemoautotrophic nocardiform bacteria from the environment, especially associated with fossil fuel, and from leprosy patients, and also from cases of "animal leprosy."

This is an ingenious hypothesis but cannot be sustained. It ignores modern information about the leprosy bacillus (responsible for infections in man, and rare infections in nine-banded armadillos and some species of monkeys), namely, that it is a mycobacterium, quite different from the unfortunately named "rat-leprosy bacillus," and quite different from nocardias. Its origins remain obscure but are unlikely to be connected with soil nocardias.

The proposed correlation suffers from the different bases for the collection of data on fuel and leprosy, and from the failure to distinguish between fuel that is exposed, in the geological sense, and that which is buried and only contacts humans when exploited. It breaks down in detail: for example, the "fossil-fuel pocket" incriminated in the outbreak of leprosy in 19th century Norway is separated from that country by hundreds of kilometers of sea and deep seabed sediments. The hypothesis cannot ac-

count for the disappearance of leprosy in western Europe at a time when exploitation of fossil fuel reached a peak, nor for the apparent absence of leprosy in the Americas until it was brought there from Europe.—P. Draper (Trop. Dis. Bull.)

Delgado Rodríguez, M., Rodríquez-Contreras Pelayo, R., Extremera Castillo, F., Serrano Ortega, S. and Gálvez Vargas, R. [Epidemiological aspects of leprosy in the province of Jaén, Spain.] Rev. Clin. Esp. **185** (1989) 99–103. (in Spanish)

Jaén is the province with the greatest number of patients with leprosy. This study analyzes the prevalence of the disease in Jaén during 1984 and 1985. At that time, all of the patients known to the health and consumer provincial delegation of Jaén were studied. The 305 patients included received a protocol aimed to study the different risk factors (age, clinical form, people living together, socioeconomic standing, ethnic, etc.). The disease has highly significant and special predilection in gypsies and it is located in a particular geographical area in the province. Leprosy did not show a rural distribution, however; it prevails in middle-sized urban nuclei. Although all patients belonged to a low social class, the disease did not show any association with the ecology and the income per inhabitant.—Authors' English Abstract

George, K., John, K. R., Muliyil, J. P. and Joseph, A. The role of intrahousehold contact in the transmission of leprosy. Lepr. Rev. **61** (1990) 60–63.

This study examines the role of intrahousehold contact in the transmission of leprosy using the case control methodology. The study was done in the leprosy control area of the Community Health and Development (CHAD) Programme of the Christian Medical College [India]. Three age-, sex- and village-matched controls were selected for each case. This study shows that persons with intrahousehold contact with leprosy have a higher risk of acquiring leprosy compared with those who did not (relative risk = 2.509; 95% confidence limits 1.23–5.109).—Authors' Summary

Irgens, L. M., Melo Caeiro, F. and Lechat, M. F. Leprosy in Portugal 1946–80: epidemiologic patterns observed during declining incidence rates. Lepr. Rev. **61** (1990) 32–49.

Compulsory notification of leprosy in Portugal formed the basis for the establishment of a national patient registry used in an epidemiological study. Highest incidence rates were observed in the coastal counties in the middle of Portugal and particularly in the municipalities with a high annual rainfall. Peak incidence rate in males was observed at the age of 25–29 years against 50–59 in females. A continuous and increasing decline in incidence rates was observed throughout the observation period, 1946–1980. Toward the end of the period the slopes of the incidence curves seemed to be identical with those observed in other countries where leprosy has previously been eradicated. This is consistent with the notion that toward the end of an endemic situation no new transmission of the disease occurs, and the incidence curve takes the shape of the right part of the distribution of incubation periods which apparently is uniform in leprosy, irrespective of time and place. The pattern observed in other areas having declining incidence rates, of an increase in age at onset by year of onset together with a lack of increase in age at onset by year of birth, was confirmed by the Portuguese data, also consistent with a break in the transmission of the disease a long time before the final termination of the endemic situation.—Authors' Summary

Kartikeyan, S., Chaturvedi, R. M. and Deo, M. G. The sociocultural dimension in leprosy vaccine trials. Lepr. Rev. **61** (1990) 50–59.

This paper briefly describes organizational, operational, and sociocultural aspects of the phase-III clinical trials of the ICRC antileprosy vaccine in Maharashtra, India. Our experience is that vaccine trials can be launched quickly and more cost effectively by using the services of health personnel from the existing public health infrastructure. That is why the trials could be launched in just 4 months after receiving the financial grant from the Indian Council of Medical

Research, New Delhi (India). At the community level, a person-to-person approach in health education scores over audio-visual aids and the mass media. The compliance in target groups is increased when preventive programs are backed up by curative services and when their privacy and daily routine are not disturbed.—Authors' Summary

Le Kinh Due. Experience in leprosy control in a developing country: Vietnam. Acta Leprol. (Genève) **7** (1989) 69–72.

In short, leprosy eradication as well as MDT implementation has started at focal points with different sizes (village, groups of villages, districts, provinces, etc.) belonging to various localities of the country (Leopard Skin). It will be expanded step by step to other points to cover one-third of the population in 1990, two-thirds in 1995 and the whole nation by the year 2000. At that date, leprosy is expected to become no more a social problem in Vietnam.—(*From the* Article)

Lombardi, C., Junqueira, T. B. and Gardia, M. R. de A. [Detection of hanseniasis in the city of Maringá, Paraná, Brazil, 1977–1988.] Hansenol. Int. **13** (1988) 1–12. (in Portuguese)

This study was dedicated to the analysis of the behavior of the Hansen's disease in Maringá, State of Paraná. Three hundred forty-eight clinical-epidemic cards were studied in patients who had Hansen's disease in the period from 1977 to 1986. It was verified that the clinical forms Virchowian + borderline had the highest percentual and prevailed in the age limit where people are economically active, concluding the same of a previous study which was performed by Belda and Lombardi and by Asseis, *et al.* The tables and figures of the disease distribution are presented according to sex, origins, year of the detection, clinical form, age when it was diagnosed (the disease), time when symptoms appeared until the diagnosis with short comments, and its importance to the epidemic valuation of Hansen's disease in this city.—Authors' English Abstract

Moreira, T. and Almedia, J. [Epidemiological study of the slum Vila Nova in the city of Duque de Caxias, Rio de Janeiro State, July 1988.] Hansenol. Int. **13** (1988) 13–20. (in Portuguese)

Due to the high endemicity of Hansen's disease in the "Duque de Caxias Municipality," Rio de Janeiro State, Brazil, an epidemiological survey of "Vila Nova" slum was planned. The goal was to verify the prevalence of skin diseases of sanitary concern and, also, to analyze the socioeconomic conditions of the population, especially those related with environmental sanitation; 45% of the dwellings were visited and 86% of the population (1903 persons) were screened by dermoneurological examination performed by auxiliary personnel. Tables show the frequency of skin diseases that received treatment on a domiciliary basis; 19 cases were suspicious of Hansen's disease. The social profile was assessed through the study of several parameters of the population: economical, sanitation, personnel, and home hygiene.—Authors' English Abstract

Pan, Y.-L., *et al.* [Possibility of leprosy eradication in Shandong Province by year 2000.] China Lepr. J. **5** (1989) 121–125. (in Chinese)

Shandong province, with a population of 77 million, was one of the provinces with a relatively higher prevalence of leprosy. Since the inauguration of the leprosy control program in 1955, a total of 52,732 leprosy patients have been registered, of which 38,238 have been cured and 1461 patients remained by the end of 1986. The detection, incidence, and prevalence rates have declined by 96.3%, 94.6%, and 95.1%, respectively, and the incidence in the 0–14 age group has declined by 98.6%.

The average relapse rate was 3.78/1000 person years, and it declined with the progress of the leprosy control program. It is estimated that the incidence will decline to less than 0.04/100,000 and prevalence to less than 0.25/100,000 by 2000 A.D. From 1955 to 1984, the incidence in 53 counties had attained the national goal of basic leprosy elimination (incidence is less than 0.2/100,000) among the total of 137 counties.

The prevalence in 69 counties had declined to less than 1/100,000 in 1986. The prevalence of leprosy in Wulian County, which has the highest in the province, will decline to less than 0.75/100,000 by the year 2000. With the implementation of multidrug therapy and other control measures, we are confident that Shandong will reach the national goal of basic elimination of leprosy by the end of the century.—Authors' English Abstract

Shao, K.-W., *et al.* [Trends of epidemics of leprosy in Fujian Province.] China Lepr. J. **5** (1989) 125–129. (in Chinese)

In Fujian Province, 27,506 cases of leprosy were found during the last 30 or more years. By the end of 1986, 1823 active cases remained, with a prevalence of 0.06/1000, showing a decrease of 90.3% from the previous high. The case-finding rate decreased by 91%. The mean incidence decreased by 87.8%. The incidence of child cases decreased significantly from 2.13/100,000 to 0.09/100,000. The mean age of the patients at the time of confirming the diagnosis shifted to the older groups, denoting that the disease was well under control. In view of the shortening of the duration of the disease, the increasing of the percentage of the patients in early stages (from 28.3% to 52.8%), the decreasing of the cases with disability and deformity (from 31.2% to 18.4%), and the increasing of the percentage of the paucibacillary cases with a single skin lesion (from 9.4% to 16.5%) in this province, a significant accomplishment in leprosy control has been achieved, and the goal of basically eradicating the disease by 1995 should be reached.—Authors' English Abstract

Todd, J. R., West, B. C. and McDonald, J. C. Human leukocyte antigen and leprosy: study in northern Louisiana and review. Rev. Infect. Dis. **12** (1990) 63–74.

We examined the relationship of human leukocyte antigen (HLA) phenotype to leprosy in six sporadic cases in northern Louisiana (U.S.A.) and in the world literature through pooling of the results of several studies. We found that HLA antigens DR2 and DQwl were associated with leprosy in the six cases in northern Louisiana (relative risks, 4.57 for DR2 and 4.53 for DQwl), but the results are not statistically significant. We pooled the Louisiana study and other population studies of HLA and leprosy. The results of the pooling show DR2 and DQwl to be associated with leprosy (relative risks, 2.65 for DR2 and 2.73 for DQwl), and these associations are highly statistically significant ($p < 1 \times 10^{-8}$ for DR2 and $p = 3.6 \times 10^{-8}$ for DQwl). Further, we pooled studies of lepromatous leprosy patients versus controls and studies of tuberculoid leprosy patients versus controls and found that DR2 and DQwl are associated with both the lepromatous and the tuberculoid forms of leprosy and that these associations are statistically significant. We consider the associations of DR2 and DQwl in these population studies to be evidence for an HLA-associated genetic influence on susceptibility to leprosy.—Authors' Abstract

Vigneron, E. The epidemiological transition in an overseas territory: disease mapping in French Polynesia. Soc. Sci. Med. **29** (1989) 913–922.

During the last 200 years in French Polynesia the people have experienced several dramatic changes in the pathological scene. First, the discovery of Tahiti and the surrounding islands at the end of the 18th century caused the spread of diseases previously unknown, usually in the form of epidemic outbreaks. In contrast, from the 1860s to soon after the end of the Second World War, health amelioration in French Polynesia was slowly occurring. This constituted a first epidemiological transition in which infectious disease mortality was sharply reduced. The distribution of vaccines, hygiene education and legislation stemmed the long period of some 100 years of demographic disaster and at last the population was able to increase. However, for a long time infectious or parasitic diseases remained the main causes of morbidity and mortality. Only from the end of the 1950s has the situation evolved to the present state where morbidity and mortality of the circulatory system and cancer are similar in

prevalence to industrialized countries. Diachronistic mapping of some of the most noteworthy diseases is presented to illustrate this last and most important phase of the epidemiological transition.—Author's Abstract

Rehabilitation

Antia, N. H. Plastic footwear for leprosy. Lepr. Rev. **61** (1990) 73–78.

The anesthetic foot in leprosy poses the most major problem in the rehabilitation of its patients. Various attempts have been made to produce protective footwear, such as the microcellular rubber-car-tire sandals. Unfortunately, these attempts have had little success on a large scale because of the inability to produce them in large numbers and the stigma attached to such unusual footwear. While such footwear may be superior to the "tennis" shoe in protecting the foot from injury by the penetration of sharp objects, it fails to distribute the weight-bearing forces which is the major cause of plantar damage and ulceration in the anesthetic foot. This can be achieved by providing rigidity to the sole, as demonstrated by the healing of ulcers in plaster of Paris casts or the rigid wooden clog. A new type of molded plastic footwear has been evolved in conjunction with the plastic footwear industry which provides footwear that can be mass produced at a low price and which overcomes the stigma of leprosy. Controlled rigidity is provided by the incorporation of a spring steel shank between the sponge insole and the hard-wearing plastic sole. Trials have demonstrated both the acceptability of the footwear and its protective effects as well as its hard-wearing properties.—Author's Summary

Ashamalla, L. Impact of leprosy on the family and intimate relationships. Chemioterpia **6** Suppl. 2 (1987) 356–357.

Leprosy is not transmitted through sexual relations. Close contact is not the main cause for its spread. The vast majority of leprotic husbands do not transmit the disease to their wives, and the reverse is also true. I can assure that many leprotic cases can live a happy marital life. Encouraging and pushing leprotic patients toward intermarriage is counter to the freedom of every individual to choose his other partner. I agree with Dr. Frist in this opinion that in order to create healthy future attitudes toward leprosy, we must stop segregation policies and the breaking up of families. We should consider leprosy a disease like any other.—Author's Conclusion

Becx-Bleumink, M., Berhe, D. and 'T Mannetje, W. The management of nerve damage in the leprosy control services. (Editorial) Lepr. Rev. **61** (1990) 1–11.

The provision of MDT should be given top priority in the control of leprosy. Early effective chemotherapy will also prevent the development of disabilities in many new patients. This is an indirect effect of antileprotic treatment. At present there are several million people in the world who are disabled due to leprosy. To the patients, their families, as well as to the general public, deformities have often much more significant implications than the infection itself. Deformities may lead to stigmatization and ostracism. If resources are available, chemotherapy and care for established deformities may be given concurrently. For the individual patients these two aspects are often inseparable. In our opinion there is no room for rehabilitation services without effective treatment, i.e., MDT. One may argue whether there is a justification for the treatment of established deformity, including the care of ulcers and reconstructive surgery, in the absence of such care for patients with deformities due to other diseases. We think that the rehabilitation services for leprosy patients should be an integral part of the general rehabilitation services in which all patients with deformities are included. The prevention of disability and deformity is, however, certainly an integral part of the

leprosy control services. One aspect in the prevention of disability is the early detection and appropriate treatment of reactions which involve the nerves. Another means of control of disability is health education which is aimed at the promotion of self-care by patients. This editorial concentrates on how the following two aspects can be handled by the leprosy control services: the early detection and appropriate treatment of nerve damage and reaction; and the prevention of increase of disability in patients with irreversible nerve function loss before secondary complications have occurred.—(*From the* Editorial)

Bell-Krotoski, J. "Pocket filaments" and specifications for the Semmes-Weinstein monofilaments. J. Hand Ther. **3** (1990) 26–31.

Despite numerous advantages, in its present form the traditional Semmes-Weinstein (S-W) monofilament kit can be improved to increase its reliability in clinical testing. This paper explores some of the recent information that has surfaced regarding the test, clarifies some physical characteristics of the material used to make the test, and suggests ways the instrument can be improved to be more useful. It underscores the need for future clinical studies to identify filament diameters, material used to make the filament, and source of material, so that variables from these can be considered or eliminated in overall clinical findings. These variables can affect the validity of clinical results.

The complete kit in its present form can be cumbersome to carry, time-consuming to use, and expensive. The complete kit is no more sensitive than the mini-kit, and clinicians should feel free to use the latter without feeling they are sacrificing the sensitivity possible with the former. The expense of the instrument is related in large part to its limited use; its production cost could be reduced were there enough demand for the instrument to be produced in large quantities. The mini-kit filaments could be placed in one instrument with colored filaments, which would make them easier to use and would eliminate the possibility of filaments being inadvertently switched. Coloring of the filament material to identify progressive diameters and forces could help toward the design of a smaller instrument that could be carried in a pocket.—Author's Summary

Breger-Lee, D., Bell-Krotoski, J. and Brandsma, J. W. Torque range of motion in the hand clinic. J. Hand Ther. **3** (1990) 7–13.

Goniometric measurement of passive range of motion is a classic evaluation tool of hand therapy, yet there is no objective quantification of the torques applied from one measurement to the next, or position of the proximal joints during measurement. This paper discusses a method of controlled torque and joint position that has been used experimentally and clinically as a tool for objective assessment of passive range of motion. Case illustrations demonstrate the usefulness of this technique in clinical case management.—Authors' Abstract

Girdhar, M., Arora, S. K., Mohan, L. and Mukhija, R. D. Pattern of leprosy disabilities in Gorakhpur (Uttar Pradesh). Indian J. Lepr. **61** (1989) 503–513.

Out of 514 leprosy cases studied, 229 (44.56%) had disability. Disability was most commonly seen in lepromatous leprosy. There was an increasing trend in disability with increasing age of patient and duration of disease. The disability rate was higher in males as compared to females. Nerve thickening and reactional states were more common in disabled cases. A dapsone-treated group showed a disability rate of 63.8% as compared to 30.0% in an untreated group. The hand was the most commonly affected site and mobile claw hand was the single most common disability. The overall disability index—D.I.—was 1.25 and lepromatous cases had highest D.I. (1.89). Disability index was higher in males and was found to increase with increasing age of patient and duration of disease.—Authors' Abstract

Nagano, J., Tada, K., Masatomi, T. and Horibe, S. Arthropathy of the wrist in leprosy—what changes are caused by long-standing peripheral nerve palsy? Arch.

Orthop. Trauma Surg. **108** (1989) 210–217.

A radiographical screening study of 338 leprotic patients was performed. Clinically, according to a nerve score (NS) designed by us, 12.9% of 674 hands showed mild nerve palsy (NS 5 or 6), 75.9% moderate (NS 3 or 4), and 11.4% severe (NS less than 2). Twenty-nine hands of 26 patients (NS 4.2 on average) demonstrated abnormal changes of the wrist joint on radiographs. We classified them into four groups: a) lunate collapse (4 patients), b) scaphoid nonunion (8), c) scaphoid cyst (3), and d) trapezium OA (11). In the lunate collapse and the scaphoid nonunion groups, destructive and reconstructive changes as described by Eichenholtz were identified on plain film. These groups demonstrated remarkable instability of the stress and dynamic roentgenograms. In contrast, the scaphoid cyst and trapezium OA wrists showed neither fracture nor instability and fewer changes than the other two groups. We considered the destructive changes that had taken place in the lunate collapse and the scaphoid nonunion wrists to be neuroarthropathy due to long-standing nerve palsy.—Authors' Summary

Trindade, M. A. B., de Lima, F. D. and de Almeida, R. G. [Physical disabilities in hanseniasis at the time of diagnosis. I. Evaluation of the disabilities. Hansenol. Int. **12** (1987) 21–28. (in Portuguese)

The evaluation of the physical disabilities at the moment of the hanseniasis diagnosis was carried out through the clinical and epidemiological forms of the 8915 cases recorded in the state of São Paulo, Brazil, from 1981 to 1983. The records of the physical disabilities were studied by three different methods: the disabilities at their highest grade, the disabilities' grade index achieved from the arithmetic mean of the added values of the different disability grades, and the absolute disabilities frequency. The study suggested that the maximum grade was the best evaluation method of the physical disabilities at the moment of the diagnosis, being an important indicator for the evaluation of prevention efforts and of the hanseniasis control.—Authors' English Abstract

Trinidade, M. A. B., Teixeira, P. R. and de Paula, S. R. [Physical disabilities in hanseniasis at the time of diagnosis. II. An index of the evaluation of the hanseniasis control program.] Hansenol. Int. **12** (1987) 29–37. (in Portuguese)

The evaluation of physical disabilities caused by hanseniasis at the moment of the diagnosis was carried out through the clinical and epidemiological forms of the 8915 cases recorded in the state of São Paulo, Brazil, from 1981 to 1983. The resulting data showed that the disabilities' evaluation at the moment of the diagnosis is an important index for the hanseniasis control program especially when related to other variable elements involved in the diagnosis of the hanseniasis situation.—Authors' English Abstract

Tzourio, C., Henry, P., Boucher, P., Parent, M., Millan, J. and Métral, S. [Clinical and electrophysiological evidence of axonal multineuritis in lepromatous leprosy.] Acta Leprol. (Genève) 7 (1989) 51–56. (in French)

This work was undertaken because there were only a few reports on neurological aspects on lepromatous leprosy. We studied 30 patients suffering from lepromatous leprosy who, at their first visit to the Institute, had never been treated. The clinical examination included a quantitative evaluation of the neurological status following the method developed by Pearson. Motor and sensory nerve conduction velocities were measured: values of conduction velocity and distal amplitude were analyzed and compared to those of a group of 22 healthy subjects. In conclusion: a) There is a high frequency of clinical and especially electrophysiological neurological impairment. This impairment can be extremely precocious and may happen shortly after the first cutaneous signs. b) Nervous impairment is diffuse, bilateral but not homogeneous. These are characteristics of mononeuritis multiplex. Impairment is predominantly sensitive and tactile sensibility is more involved than thermo-algic sensation. c) The radial superficial nerve is the most frequently involved, clinically and electrophysiologically. d) The electrophysiological results,

showing a normal or slightly reduced conduction velocity and a low amplitude of evoked potential, are in favor of predominantly axonal damage.—Authors' English Summary

Yin, K.-M. [Follow-up of 64 leprosy patients with deformities accepting surgical operation.] China Lepr. J. **5** (1989) 188–190. (in Chinese)

The follow-up of 64 cases of leprosy patients with various deformities who accepted surgical operations 2 to 12 years earlier shows that 36 cases have had excellent results; 9 cases were better, 11 a little better, and 8 had no effect. Thirty-one of 36 footdrops have had better recovery and in one case out of those who failed to respond to the operation it was found that an implanted tendon had undergone fatty degeneration. All of five clawhands have had better recovery. The delta decurtation of palpebral fissure was found to be very good for lagophthalmos; the author regards it as simple to do, needing only a little incision, and can be used as a routine operation.—Author's English Abstract

Zhang, G.-C. and Zheng, T.-S. [Correction of lagophthalmos in leprosy by transferring of temporalis muscle bundle and fascial sling—report of 26 cases.] Chin. J. Plastic Surg. Burns **3** (1987) 14–15. (in Chinese) English abstract on page 77.

The results of surgical procedures, such as fascial sling and tarsorrhaphy, used to correct lagophthalmos were unsatisfactory. In this paper the long-term effects of temporalis muscle bundle transfer performed on 26 patients (35 eyes) are presented. The procedures and the criteria for results graded are described. From the results we have obtained, the temporalis muscle bundle transfer seems very encouraging. The authors recommend it as a routine operation for lagophthalmos.—Authors' English Abstract

Other Mycobacterial Diseases and Related Entities

Abbot, N. C., Spence, V. A., Beck, J. S., Carnochan, F. M. T., Gibbs, J. H. and Lowe, J. G. Assessment of the respiratory metabolism in the skin from transcutaneous measurements of pO_2 and pCO_2: potential for non-invasive monitoring of response to tuberculin skin testing. Tubercle **71** (1990) 15–22.

A method is described for noninvasive transcutaneous (tc) measurement of tissue respiratory gas tensions in the skin on the forearm for study of delayed hypersensitivity reactions in man. Steady state values for $tcpO_2$ and $tcpCO_2$ were measured, and the skin respiratory rate (oxygen consumption) and the tissue pH were estimated from the changes in $tcpO_2$ and $tcpCO_2$ observed after interruption of the arterial circulation by cuff occlusion for 4 minutes. The extent of within-experiment and between subject variation in the steady-state measurements was not great (coefficient of variation 10%): $tcpCO_2$.ss (steady state) was higher in men and $tcpO_2$.ss was higher in women, but the extent of these sex differences was also small. Reference ranges have been established for tc measurements and calculated indices of tissue respiration in the undisturbed forearm skin of normal volunteers, against which the changes induced by tuberculin testing can be assessed. Severe changes, indicative of profound hypoxia and acidosis, are seen in intense delayed-hypersensitivity reactions. Similar, but less severe changes were seen at the site of skin tests on BCG-vaccinated subjects who were "negative" by conventional criteria of measurement of dermal induration and they became greatly exaggerated after successful revaccination. Intradermal injection of saline did not induce hypoxia or local acidosis. These new methods are very sensitive indicators of the tissue response in the DHS reaction.—Authors' Summary

Appelberg, R., Soares, R., Ferreira, P. and Silva, M. T. Induction of non-specific im-

munosuppression in mice by mycobacterial infections and its relationship to macrophage activation. Scand. J. Immunol. **30** (1989) 165–174.

The development of nonspecific immunosuppression during the infection of different strains of mice with three mycobacterial species was evaluated by studying the immune response to a heterologous antigen (sheep red blood cells) and comparing it with the induction of nonspecific resistance to a *Listeria monocytogenes* challenge. It was shown that early (at 15 days) immunosuppression developed in *Mycobacterium avium*-susceptible mouse strains infected with a high inoculum dose [2.5×10^8 colony forming units (CFU)] of virulent *M. avium* but not in resistant mice infected with a similar inoculum nor in susceptible mice infected by a smaller inoculum dose (2.5×10^6 CFU). In the latter case it developed only during the second month of infection and was of smaller magnitude. An inoculum of *M. avium* of attenuated virulence did not induce immunosuppression. *M. lepraemurium* induced a late immunosuppression, which occurred when extensive bacterial proliferation had already taken place. The nonpathogenic *M. bovis* BCG induced immunosuppression in C57BL/6 mice. The results do not establish a correlation between the development of generalized immunosuppression and susceptibility to infection. It could be seen that the early immunosuppression was observed in those situations where there was extensive macrophage activation as shown by the development of nonspecific resistance to a listeria challenge. The late immunosuppression was observed when bacterial proliferation was extensive.—Authors' Abstract

Barnes, P. F., Mehra, V., Hirschfield, G. R., Fong, S.-J., Abou-Zeid, C., Rook, G. A. W., Hunter, S. W., Brennan, P. J. and Modlin, R. L. Characterization of T cell antigens associated with the cell wall protein-peptidoglycan complex of *Mycobacterium tuberculosis*. J. Immunol. **143** (1989) 2656–2662.

Mycobacterium tuberculosis cell walls are likely to contain critical T-cell antigens (Ag) capable of inducing protective immunity against the development of tuberculosis in animal models. Therefore, we characterized cell-wall-associated Ag that stimulate T lymphocytes in tuberculosis patients and clinically well tuberculin-positive individuals. A protein-peptidoglycan complex isolated from the *M. tuberculosis* cell wall had potent immunologic activity, evoking PBMC proliferative responses similar to those induced by sonicated whole *M. tuberculosis*. In order to characterize the immunoreactive protein determinants associated with the protein-peptidoglycan complex, T-cell lines were established to cell-wall Ag and used to probe *M. tuberculosis* proteins separated by SDS-PAGE. These T-cell lines proliferated primarily to protein Ag of 10, 19, 23, 28, 30, 40 to 50, and 65 kDa. Cell-wall-reactive T-cell clones that recognized the 10-, 23-, 28-, and 30-kDa proteins as single bands on SDS-PAGE did so under reducing and nonreducing conditions, suggesting that these are not proteolytic fragments or subunits of larger protein aggregates. We propose that these protein monomers, when post-translationally complexed with peptidoglycan, are the key ingredients of the immunogenic protein-peptidoglycan complex. In order to assess the relationship of the cell-wall-associated Ag to those secreted proteins from "early culture filtrates" of actively growing *M. tuberculosis* recently implicated in eliciting protective immunity, cell-wall-reactive T-cell clones were tested for their ability to recognize early culture filtrates. Results revealed that at least three proteins shared with the cell-wall complex are contained within early culture filtrates. Our data indicate that antigenic determinants associated with the protein-peptidoglycan complex of the *M. tuberculosis* cell wall may be involved in protective immunity and hence are potential candidates for inclusion in an effective antituberculosis vaccine.—Authors' Abstract

Bermudez, L. E. M., Stevens, P., Kolonoski, P., Wu, M. and Young, L. S. Treatment of experimental disseminated *Mycobacterium avium* complex infection in mice with recombinant IL-2 and tumor necrosis factor. J. Immunol. **143** (1989) 2996–3000.

Mycobacterium avium complex (MAC) is the most common bloodstream pathogen isolated from patients with AIDS. We have previously shown that TNF alone or in combination with IL-2 can activate human and murine macrophages *in vitro* to kill MAC strains isolated from disseminated infections. To determine whether treatment with TNF and IL-2 could effect the course of disseminated MAC infections in a murine model of disseminated MAC infection, we infected C57BL mice with 3×10^8 bacteria i.v. and 1 wk later administered: 1) IL-2, 100 μg/kg; 2) TNF, 25 μg/kg; 3) IL-2, 50 μg/kg, and TNF, 12.5 μg/kg; and 4) saline. IL-2 was injected i.p. daily with TNF being administered in cycles of 3 out of 4 consecutive days. Fourteen days after starting therapy, blood was cultured and mice were sacrificed for quantitative cultures of liver and spleen homogenates. IL-2, TNF, and IL-2/TNF treated groups showed an 87 ± 5%, 57 ± 9%, 88 ± 6% decrease in bacteremia ($p = 0.05$ for TNF-treated animals and < 0.04 for the other two groups, compared with control). The combination IL-2/TNF was the only treatment that showed a trend toward an absolute decrease in the number of bacteria in the blood. Reduction in colony counts of liver and spleen were 77 ± 4% and 87 ± 6%, respectively, for treatment with IL-2, 58 ± 7% and 87 ± 5% for TNF, and 60 ± 10% and 82 ± 6% for IL-2/TNF, respectively. These results suggest that both cytokines may play a role in the control of *M. avium* infection and that the combination of a half-dose of IL-2 and TNF, despite not showing any greater efficacy, can be less toxic than TNF or IL-2 alone and might be useful for the therapy of disseminated infection.—Authors' Abstract

Bermudez, L. E. M. and Young, L. S. Oxidative and non-oxidative intracellular killing of *Mycobacterium avium* complex. Microb. Pathog. **7** (1989) 289–298.

Among mycobacteria, those belonging to the *Mycobacterium avium* complex (MAC) are the most commun cause of bacteremia in AIDS patients. To understand better the mechanisms by which human macrophages kill intracellular MAC, we studied in an *in vitro* test system transparent morphotypes of the three most common bacteremic serotypes from AIDS patients and an opaque variant, obtained *in vitro* from the most mouse-virulent strain (MAC 101). The three serotypes differed in susceptibility to oxidative bactericidal mechanisms of macrophages. The transparent morphotype of strain 101 (serotype 1) was completely resistant to the intracellular killing effects of a phagocyte's reactive oxygen radicals and hydrogen peroxide; whereas strains 109 (serotype 4), 100 (serotype 8), and the opaque variant from strain 101 were killed by oxidative bactericidal mechanisms. However, even for these bacteria, nonoxidative mechanisms appear to have a role in intracellular killing.—Authors' Abstract

Borremans, M., de Wit, L., Volckaert, G., Ooms, J., de Bruyn, J., Huygen, K., van Vooren, J.-P., Stelandre, M., Verhofstadt, R. and Content, J. Cloning, sequence determination, and expression of a 32-kilodalton-protein gene of *Mycobacterium tuberculosis*. Infect. Immun. **57** (1989) 3123–3130.

We describe the identification of the gene encoding an immunodominant 32-kilodalton (kDa) protein of *Mycobacterium tuberculosis*. The 32-kDa antigen is abundantly secreted into the culture supernatant of a variety of mycobacteria and appears to be a major stimulant of cellular and humoral immunity against mycobacteria. Recombinant clones expressing a 140- or 125-kDa β-galactosidase fusion protein reactive with rabbit polyclonal anti-32 kDa protein serum were detected. The corresponding DNA sequence contains a 1008-base-pair coding region. The deduced amino-acid sequence corresponds to a 336-residue protein including the previously determined NH_2-terminal sequence of the 32-kDa protein. Upstream of this NH_2-terminal region, the gene codes for a signal peptide required for the secretion of a 294-amino-acid-long mature protein. A putative promotor sequence would be located upstream of the open reading frame. Comparison of the *M. tuberculosis* 32-kDa antigen with the *M. bovis* BCG α-antigen revealed 73.8% homology between DNA sequences and 72.8% homology between amino-acid sequences (signal and mature protein). Finally, the 140-kDa fusion protein could selectively be recog-

nized by human tuberculous sera. This result confirms our previous finding that the 32-kDa antigen could be a valuable tool for the serological diagnosis of tuberculosis. Moreover, the availability of recombinant proteins opens perspectives for the localization of relevant B- and T-cell epitope regions on the 32-kDa antigen.—Authors' Abstract

Brisson-Noël, A., Gicquel, B., Lecossier, D., Lévy-Frébault, V., Nassif, X. and Hance, A. J. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. Lancet **2** (1989) 1069–1071.

A method based on DNA amplification and hybridization for the rapid detection of *Mycobacterium tuberculosis* was used to test 35 clinical specimens (sputrum, gastric aspirate, abscess aspirate, biopsy sample) from 34 patients in whom tuberculosis was suspected. *M. tuberculosis* was detected in 15 specimens, 2 of which were negative by standard microbiological criteria (microscopy and/or culture); 20 specimens, negative by standard methods, were also negative by the amplification method. *M. tuberculosis* was also detected in peripheral blood samples of 2 of 4 patients with AIDS from whom the organism had been isolated.—Authors' Summary

Colebunders, R. L., Lebughe, I., Nzila, N., *et al.* Cutaneous delayed-type hypersensitivity in patients with human immunodeficiency virus infection in Zaire. J. AIDS **2** (1989) 576–578.

Cutaneous anergy occurred in 23 (46%) of 50 asymptomatic HIV-seropositive patients compared with none of 34 healthy controls (hospital workers) and was most prevalent in those with advanced HIV infection. HIV-seropositive patients with a positive tuberculin reaction were more likely to have active tuberculosis than HIV-seropositive patients with a negative tuberculin reaction, and the authors conclude that a tuberculosis diagnosis should be pursued in symptomatic HIV-seropositive patients with a positive tuberculin test.—H. Richardson (Trop. Dis. Bull.)

Collins, T. and Levett, P. N. Radiometric studies on the use of selective inhibitors in the identification of *Mycobacterium* spp. J. Med. Microbiol. **30** (1989) 175–181.

Radiometric selective inhibition tests were developed and evaluated for the rapid differentiation of *Mycobacterium* spp. Both a ρ-nitrobenzoic acid (PNB) test and a commercially prepared ρ-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test successfully differentiated *M. tuberculosis* and *M. bovis* from "atypical" mycobacteria or mycobacteria other than tubercle bacilli (MOTT). Thiophene-2-carboxylic acid hydrazide (TCH) readily distinguished human *M. tuberculosis* strains from *M. bovis,* irrespective of resistance to isoniazid. Both PNB and TCH tests were utilized in a routine radiometric susceptibility testing scheme over a period of 1 year in which 110 isolates of *M. tuberculosis,* 10 of *M. bovis* and 1 isolate of BCG were correctly differentiated from 10 isolates of MOTT. The rapidity, sensitivity and specificity of these radiometric tests can play a useful role in mycobacterial identification.—Authors' Summary

Conlon, C. P., Banda, H. M., Luo, N. P., Namaambo, M. K. M., Perera, C. U. and Sikweze, J. Faecal mycobacteria and their relationship to HIV-related enteritis in Lusaka, Zambia. AIDS **3** (1989) 539–541.

The prevalence of infection with mycobacteria, both typical and atypical, is increasing along with prevalence of infection with HIV. Patients with pulmonary tuberculosis (PTB) and patients with chronic diarrhea are forming a growing proportion of the patient population in hospitals in central Africa. To investigate the possibility that mycobacteria may be responsible for some of the HIV-related enteropathy seen in Lusaka, we studied 89 patients in four different diagnostic groups, clinically, by Mantoux test and by microscopy and culture of stool specimens for mycobacteria. In the HIV-positive group with chronic diarrhea (N = 31), 2 patients were found to have mycobacteria on fecal smear and 3 were culture positive while of the 15 HIV-negative controls, 3 were smear positive and 3 were cul-

ture positive. Of the 15 patients with proven PTB, 3 had positive fecal smears but 0 were culture positive. In the fourth group of 24 patients with suspected PTB, 7 were smear positive and 5, culture positive. Only in this last group was there some correlation between smear results and culture results. Although this last finding is difficult to explain, it appears that there is no correlation between the symptom of chronic diarrhea and the presence of mycobacteria in the stool. We conclude that mycobacteria do not play a significant role in the pathogenesis of HIV-related enteropathy in Lusaka.—Authors' Abstract

DeJoy, S. Q., Ferguson, K. M., Sapp, T. M., Zabriskie, J. B., Oronsky, A. L. and Kerwar, S. S. Streptococcal cell wall arthritis; passive transfer of disease with a T cell line and crossreactivity of streptococcal cell wall antigens with *Mycobacterium tuberculosis.* J. Exp. Med. **170** (1989) 369–382.

Primary lymph node cells derived from streptococcal cell wall arthritic rats or those derived from adjuvant arthritic rats proliferated in response to cell-wall antigens derived from either streptococcal cell walls or those from *Mycobacterium tuberculosis.* In addition, two T-cell lines have been isolated from lymph nodes of rats during the chronic phase of streptococcal cell-wall arthritis. These T-cell lines transferred clinical disease to naive syngeneic irradiated recipients, and they proliferated in the presence of cell-wall antigens derived from streptococci or antigens derived from mycobacterium but failed to proliferate in the presence of the 65-kDa antigen (containing the sequence TFGLQLELT) derived from mycobacterium. These observations indicate that T cells play a crucial role in the pathogenesis of streptococcal cell-wall arthritis and suggest that antigenic crossreactivity exists between cell walls of group A streptococci and antigens derived from mycobacterium. The 65-kDa mycobacterium protein is not involved in the observed antigenic crossreactivity.—Authors' Summary

Douglas-Jones, A. G., Duddridge, L. R. and Jenkins, P. A. Killing of *Mycobacterium tuberculosis* in tissue by microwaves with simultaneous tissue fixation. Tubercle **71** (1990) 7–13.

Guinea-pig liver heavily infected with *Mycobacterium tuberculosis* has been sterilized by exposure to microwaves in a standard commercially available domestic oven. Subsequent histology showed good tissue preservation and organisms of normal morphology were identified by Ziehl-Neelsen staining. The findings allow the safe use of frozen sections for diagnosis in tissue containing *M. tuberculosis.*—Authors' Summary

Espita, C., Cervera, I., González, R. and Mancilla, R. A 38-kD *Mycobacterium tuberculosis* antigen associated with infection; its isolation and serologic evaluation. Clin. Exp. Immunol. **77** (1989) 373–377.

To identify antigens that could be specifically associated with tuberculosis infection, the antibody response to *Mycobacterium tuberculosis* antigens of patients with pulmonary tuberculosis and of healthy individuals were compared by immunoblot. In healthy individuals, serum antibodies were found in the majority of cases. Bands of 60 and 32–31 kilodaltons (kDa) were the antigens more frequently recognized by antibodies of normal sera (55.8% and 64.7%, respectively). In patients with pulmonary tuberculosis, the number and intensity of the developed antigen bands were much higher than in normal individuals. Antigens reacting preferentially with tuberculosis sera were also identified. Furthermore, a unique disease-associated protein antigen of 38 kDa was found to react with 57% of patients' sera but with none of the controls. This antigen was isolated by elution from nitrocellulose membranes and tested as an ELISA reagent in the serodiagnosis of pulmonary tuberculosis. A specificity of 0.96 and sensitivity of 0.68 were obtained.—Authors' Summary

Espita, C. and Mancilla, R. Identification, isolation and partial characterization of *Mycobacterium tuberculosis* glycoprotein antigens. Clin. Exp. Immunol. **77** (1989) 378–383.

In *Mycobacterium tuberculosis* culture filtrates, three concanavalin A (ConA)-binding bands of 55, 50 and 38 kilodaltons (kDa) were identified by labelling blotted proteins with a ConA-peroxidase conjugate. Binding was inhibited by the competitor sugar α-methyl mannoside and by reduction with sodium *m*-periodate. Bands of 55, 50 and 38 kDa stained with Coomassie blue were sensitive to digestion with proteases, thus indicating that they are proteins. Glycoproteins were isolated by lectin-affinity chromatography or by elution from nitrocellulose membranes. On the isolated form, the 55–50-kDa doublet glycoprotein was 65.4% protein and 34.6% sugar. The purified 38-kDa molecule was 74.3% protein and 25.7% carbohydrate. By immunoblot, antibodies against mycobacterial glycoproteins were demonstrated in immunized rabbits and in patients with pulmonary tuberculosis, but not in healthy individuals. Treatment with sodium *m*-periodate abolished binding of rabbit antibodies to the 38-kDa glycoprotein. Reactivity of the 55–50-kDa doublet glycoprotein was not altered by reduction. By immunoblot with monoclonal antibodies TB71 and TB72, a carbohydrate-dependent and a carbohydrate-independent epitope could be identified on the 38-kDa glycoprotein.—Authors' Summary

Evans, A. T., Croft, S. L., Peters, W. and Neal, R. A. Antileishmanial effects of clofazimine and other antimycobacterial agents. Ann. Trop. Med. Parasitol. **83** (1989) 447–454.

In the search for more effective alternatives to the presently used antileishmanial drugs, the activity of the major groups of antimycobacterial compounds has been examined, both *in vitro* and in animal models of infection. *In vitro,* clofazimine was the most active compound tested, with a mean ED_{50} of 2.3 mgl^{-1} against *Leishmania mexicana amazonensis,* 1.4 mgl^{-2} against *L. donovani* and 0.5 mgl^{-1} against *L. major.* Other active compounds were the thiosemicarbazone, thiambutosine, and salinazid, a derivative of isoniazid. Isoniazid itself was inactive, and rifampin only partially active. *In vivo,* only clofazimine displayed significantly activity, and it was most effective against the cutaneous infections. It is concluded that antimycobacterial activity is in general a poor predictor of antileishmanial potency.—Authors' Abstract

Gangadharam, P. R. J., Perumal, V. K., Jairam, B. T., Podapati, N. R., Taylor, R. B. and LaBrecque, J. F. Virulence of *Mycobacterium avium* complex strains from acquired immune deficiency syndrome patients: relationship with characteristics of the parasite and host. Microb. Pathog. **7** (1989) 263–278.

The virulence of 24 strains of *Mycobacterium avium* complex (MAC) isolated from patients with acquired immune deficiency syndrome (AIDS) was assessed using the beige mouse model. Most changes in colony forming unit (cfu) counts in spleen and lungs, and spleen weights occurred between days 1 and 14, with comparatively smaller changes 14–28 days postinfection. The virulence was assessed by a score formulated from the four most useful parameters: mortality, spleen cfu, lung cfu, and spleen weights at 28 days. The scores of the 24 strains showed a normal distribution; 4 strains falling above one standard deviation from the mean were classified as high virulent, those 4 falling below one standard deviation as low virulent, and the remaining 16 as of intermediate virulence. Virulence was associated with the total number of plasmids and the occurrence of large plasmids (> 100 MDa) in the MAC strains. There was an inverse correlation between virulence and the organism's capacity to trigger the release of oxygen metabolites from peritoneal macrophages. Macrophages from mice infected with the MAC strains of different degrees of virulence released superoxide anion (O_2^-) with a peak at 2 weeks, the peak levels bearing an inverse correlation to virulence. No association was seen between virulence and source of specimens, biochemical characteristics, drug susceptibility, serotypes or phage types.—Authors' Abstract

Gilburd, B. S., Markov, A. N., Fondaminskaya, L. D. and Avdienko, V. G. [Antituberculous antibodies to antigens of different molecular weights in patients with active tuberculosis of the lungs.] Probl. Tuberk. **11** (1989) 40–43. (in Russian)

Antibodies were determined in 72 tuberculous patients and 27 healthy donors using enzyme immunoassay (EIA) and immunoblotting (IB). It was shown that the levels of the antibodies to BCG and H37Rv sonicates were lower in the patients with disseminated tuberculosis than in the patients with fibrocavernous or infiltrative tuberculosis. Significant differences in the antibody spectra in the patients with different forms of tuberculosis were also shown. Thus, in the patients with disseminated tuberculosis IB with BCG and H37Rv sonicates revealed only single bands in the region of the reaction with the antigens of low molecular weights. In the patients with infiltrative tuberculosis the reaction with H37Rv or BCG sonicates most frequently revealed the antibodies to the antigen determinants with molecular weights of 50, 30, 41, 54, 52, and 60.43 or 45.5, 40.5, 57 and 52 kDa, respectively. In the patients with fibrocavernous tuberculosis, the reaction with H37Rv or BCG sonicates most frequently revealed the antibodies to the antigen determinants with molecular weights of 54, 42, 48, 30, and 13.5 or 24, 57, 37 and 14.5 kDa, respectively.—Authors' English Abstract

Grange, J. M. and Davey, R. W. Antibacterial properties of propolis (bee glue). J. R. Soc. Med. **83** (1990) 159–160.

Propolis (bee glue) was found to have antibacterial activity against a range of commonly encountered cocci and gram-positive rods, including the human tubercle bacillus, but only limited activity against gram-negative bacilli. These findings confirm previous reports of antimicrobial properties of this material, possibly attributable to its high flavonoid content.—Authors' Summary

Heifets, L. B. and Lindholm-Levy, P. J. Is pyrazinamide bactericidal against *Mycobacterium tuberculosis*? Am. Rev. Respir. Dis. **141** (1990) 250–252.

Bacterial activity of pyrazinamide (PZA) was tested at pH 5.6 in 7H12 broth against drug-susceptible *Mycobacterium tuberculosis* strains. The highest tested concentrations of PZA, 500 and 1000 μg/ml, killed no more than 76% of the bacterial population. These concentrations are more than 32 times greater than the minimal inhibitory concentration (MIC) and the achievable *in vivo* concentrations. Despite high clinical efficacy of PZA and its so-called sterilizing activity in mouse experiments, this drug is much less bactericidal *in vitro* than any other known antituberculosis drug.—Authors' Summary

Hoffner, S. E., Kratz, M., Olsson-Liljequist, B., Svenson, S. B. and Kallenius, G. *In-vitro* synergistic activity between ethambutol and fluorinated quinolones against *Mycobacterium avium* complex. J. Antimicrob. Chemother. **24** (1989) 317–324.

Ciprofloxacin, ofloxacin and norfloxacin were ineffective at clinically relevant concentrations against the *Mycobacterium avium* complex (MAC) *in vitro* as measured by radiometric respirometry. Only 2 of 30 clinical isolates of MAC were susceptible to any of the tested quinolones. By contrast good antibacterial activity was obtained when any of the quinolones was combined with ethambutol. The synergistic effect was most pronounced for the combination of ethambutol and ciprofloxacin, to which 76 of 100 strains were susceptible. It is suggested that the synergism is based on an enhanced penetration of the quinolones by ethambutol.—Authors' Abstract

Inderlied, C. B., Lancero, M. G. and Young, L. S. Bacteriostatic and bactericidal *in-vitro* activity of meropenem against clinical isolates, including *Mycobacterium avium* complex. J. Antimicrob. Chemother. **24** Suppl. A (1989) 85–99.

For clinical isolates the MICs of meropenem were significantly lower than the MICs of imipenem, especially against *Pseudomonas aeruginosa.* Furthermore, meropenem was bactericidal for *Pseudomonas* spp., including *Ps.* (*Xanthomonas*) *maltophilia,* and staphylococci, with MBCs on average only twofold above the respective MICs. The bactericidal activity was confirmed by killing curve assays. Strains with high meropenem MICs ($\geq$ 8 mg/l) were killed by a combination of the carbapenem and amikacin, with kinetics that indicated synergism between the two antimicrobial agents.—Authors' Abstract

Jeevan, A. and Kripke, M. L. Effect of a single exposure to ultraviolet radiation on *Mycobacterium bovis* bacillus Calmette-Guérin infection in mice. J. Immunol. **143** (1989) 2837–2843.

BALB/c and C3H mice were exposed on the dorsal skin to 45 kJ/m^2 of UVB radiation from FS-40 sunlamps 3 days before infection with 1×10^6 live units of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) (Tice strain) in the foot pad. At regular intervals, groups of mice were tested for a delayed-type hypersensitivity (DTH) response to the purified protein derivative (PPD) of tubercle bacilli, and the course of infection was monitored by measuring the size of the infected foot pad, enlargement of the draining lymph node, and the number of bacteria in the spleen and lymph node. In both strains the DTH response to PPD was significantly delayed in UV-treated mice compared to unirradiated mice, when tested 21 and 42 days after BCG infection. By day 50, no significant difference was detected in the DTH response between irradiated and unirradiated mice. UV treatment reduced the size of the lymph node draining the site of BCG infection in both strains of mice and the size of the infected foot pad in C3H mice but not in BALB/c mice. In both strains of mice the total number of bacteria in the spleen and the draining lymph node increased after UV irradiation. When irradiated 3, 5, 18, or 21 days after BCG infection, BALB/c mice also showed a significant decrease in their DTH response to PPD, indicating that the UV-induced suppression of BCG occurs both at the induction and the elicitation stages of the immune response. Thus, mice exposed to a single dose of UV radiation either before or after BCG infection showed an impaired DTH response to mycobacteria, which was accompanied by an increase in the multiplication of bacteria in the tissues, even though the organisms were introduced at an unirradiated site. These studies demonstrate that a systemic effect of UV irradiation can interfere with the development and expression of immunity to pathogenic bacteria in mice.—Authors' Abstract

Khardori, N., Rolston, K., Rosenbaum, B., Hayat, S. and Bodey, G. P. Comparative *in-vitro* activity of twenty antimicrobial agents against clinical isolates of *Mycobacterium avium* complex. J. Antimicrob. Chemother. **24** (1989) 667–673.

The *in vitro* susceptibility of *Mycobacterium avium* complex isolates, obtained from immunosuppressed patients with and without the acquired immunodeficiency syndrome (AIDS), to various antimicrobial agents was determined. Amikacin, the 4-quinolone compounds—ciprofloxacin, temafloxacin and PD 117558—and the penem SCH 34343 were active against most of the isolates. *In vitro* synergism using selected antimicrobial combinations could not be demonstrated. No differences in the susceptibility, depending upon the source of the isolates (AIDS or nonAIDS), were noted.—Authors' Abstract

Lecoeur, H. F., Truffot-Pernot, C. and Grosset, J. H. Experimental short-course preventive therapy of tuberculosis with rifampin and pyrazinamide. Am. Rev. Respir. Dis. **140** (1989) 1189–1193.

In a first experiment, the efficacy of a 6-month course of isoniazid (INH) alone in comparison with 2-month courses of rifampin (RMP) alone, RMP + pyrazinamide (PZA), or RMP + PZA + INH as preventive therapy of tuberculosis was evaluated in the mouse. To simulate the infected, nondiseased state of humans, a nonreplicating bacillary population of limited size was developed in the mouse. Mice were vaccinated intravenously with 2.74 $\log_{10}$ *Mycobacterium bovis* BCG and infected a month later with 3.38 $\log_{10}$ *M. tuberculosis*. Treatment began 2 wk after infection when the mean size of the *M. tuberculosis* population was 4.98 ± 0.26 $\log_{10}$ cfu in the spleen. After 2 months of therapy, the proportion of mice with positive spleen cultures was 100%, 50%, 0%, and 20% in those animals treated, respectively, with INH alone, RMP alone, RMP ± PZA, or RMP + PZA + INH. After 6 months of therapy with INH alone, the proportion of mice with positive spleen cultures was 30%. In order to confirm the extreme activity of the combination RMP + PZA and to assess the value of 3 months of therapy with RMP, a second experiment was performed following similar procedures. On completion of treatment, the proportion of

mice with positive spleen cultures was 100%, 20%, 0%, and 80% in those animals treated, respectively, for 6 months with INH alone, 3 months with RMP alone, or 2 months with RMP + PZA, or RMP + PZA + INH. Six months after the end of treatment, the respective proportions of mice with positive spleen cultures were 100%, 60%, 56%, or 95%, suggesting that 2 months of therapy with RMP + PZA or 3 months of therapy with RMP alone were more effective than 6 months of therapy with INH alone.—Authors' Summary

Lounatmaa, K. and Brander, E. Crystalline cell surface layer of *Mycobacterium bovis* BCG. J. Bacteriol. **171** (1989) 5756–5758.

A paracrystalline surface layer (S layer) was found as the outermost layer of the cell wall of five *Mycobacterium bovis* BCG strains. An oblique arrangement of the subunits in the S layer was only clearly seen in thin-sectioned and shadowed preparations, and the unit constant was about 5.5 nm.—Authors' Abstract

Lu, X.-H., *et al.* [Preliminary report on culture of *M. lepraemurium in vitro.*] China Lepr. J. **5** (1989) 139–142. (in Chinese)

Test tube culture of *Mycobacterium lepraemurium* has been successfully conducted on Ogawa medium in the authors' lab. The positive rate was 25.6% of primary isolation of *M. lepraemurium* Hawaii strain and 80.2% of secondary isolation in the first 20 passages, after which the positive rates of isolation decreased to 58.8%. Mice experimentally infected with the bacterial clones obtained from the secondary passages developed typical pathologic lesions of *M. lepraemurium.* The harvested bacilli from infected mice repeatedly grew on Ogawa medium. The advantages of this test tube culture of *M. lepraemurium* in screening test for antileprosy drugs were discussed in the paper.—Authors' English Abstract

Munk, M. E., Schoel, B., Modrow, S., Karr, R. W., Young, R. A. and Kaufmann, S. H. E. T lymphocytes from healthy individuals with specificity to self-epitopes shared by the mycobacterial and human 65-kilodalton heat shock protein. J. Immunol. **143** (1989) 2844–2849.

The immune response to mycobacterial pathogens comprises a significant percentage of T cells with specificity for a 65-kDa heat-shock protein (hsp) which is highly conserved in bacteria and man. PBMC were activated *in vitro* with killed *Mycobacterium tuberculosis* and afterward tested for CTL activity on autologous target cells primed with 1) killed *M. tuberculosis,* 2) intact recombinant 65-kDa hsp of *M. bovis/M. tuberculosis*; or 3) tryptic fragments of the recombinant 65-kDa hsp. Strong CTL activity was observed on targets primed with killed *M. tuberculosis* or with tryptic fragments of the 65-kDa hsp, but not on those primed with the intact 65-kDa hsp. *M. tuberculosis*-activated T cells from 2/13 donors tested exerted killer activity against unprimed targets. To assess whether T-cell responses were directed against self-epitopes shared by the mycobacterial and human 65-kDa hsp, four peptides of at least 10 amino acids length were synthesized corresponding to fully or almost identical regions of these molecules. Peripheral blood T cells from 8 of 9 individuals tested, after activation with killed *M. tuberculosis,* expressed strong CTL activity toward autologous targets primed with one or more of these synthetic peptides. By using HLA-DR transfected murine L cells, we found that the epitopes were recognized in the context of histocompatible HLA-DR (class II) molecules. We conclude that the demonstration of T cells with specificity to self-epitopes *in vitro* is not indicative for autoimmune disease. However, if at certain stages of infection such T cells are activated by crossreactive microbial epitopes, they could cause autoimmune responses.—Authors' Abstract

Ramesh, V., Samuel, B., Misra, R. S. and Nath, I. *In situ* characterization of cellular infiltrates in lupus vulgaris indicates lesional T-cell activation. Arch. Dermatol. **126** (1990) 331–335.

Skin biopsy specimens from nine patients with lupus vulgaris were examined *in situ* by means of monoclonal antibodies directed against phenotypes of lymphocyte subsets, Langerhans' cells, HLA-DR antigens, and interleukin 2 (IL-2) receptor. The epidermis showed prominent changes, including intense expression of HLA-DR on ker-

atinocytes, increase in epidermal cell layers, moderate-to-high Langerhans' cell hyperplasia, and infiltration by CD3+ pan-T cells as well as CD8+ (cytotoxic/suppressor) and CD4+ (helper/inducer) T cells. The predominant lymphocyte in the dermal granulomas was the activated CD3+ T cell, expressing major histocompatibility complex (MHC) class II antigens and IL-2 receptor. CD4+ and CD8+ cells were randomly distributed among the epithelioid cells, which showed intense staining for MHC class II antigens. In all except two patients, the CD4+ population was greater than that of the CD8+ cells. CD1+ Langerhans' cells were scattered in moderate numbers in the dermal granulomas. Acid-fast bacilli were conspicuously absent in the biopsy specimens. These features suggest that T-cell activation and Langerhans' cell hyperplasia are prominent features of dermal tuberculosis.—Authors' Abstract

Rivoire, B., Ranchoff, B. J., Chatterjee, D., Gaylor, H., Tsang, A. Y., Kolk, A. H. J., Aspinall, G. O. and Brennan, P. J. Generation of monoclonal antibodies to the specific sugar epitopes of *Mycobacterium avium* complex serovars. Infect. Immun. **57** (1989) 3147–3158.

Monoclonal antibodies have been generated to the unique distal sugar epitopes on the oligosaccharide haptens of the glycopeptidolipid antigens of clinically prominent members of the *Mycobacterium avium* serocomplex. Thus, antibodies are described that recognize the distal O-acetyl-α-L-rhamnopyranosyl residue of the specific glycopeptidolipid of *M. avium* serovar 1, the 4-*O*-acetyl-2,3-di-*O*-methyl-α-L-fucopyranose of serovara 2, the 4-*O*-methyl-α-L-rhamnopyranosyl-(1→4)-2-*O*-methyl-α-L-fucopyranosyl unit of serovar 4, the 4,6-(1′-carboxyethylidene)-3-*O*-methyl-β-D-glucopyranosyl unit of serovar 8 [and the 4,6-(1′-carboxyethylidene-β-D-glucopyranosyl residue of serovar 21], and the 4-*O*-acetyl-2,3-di-*O*-methyl-α-L-fucopyranosyl(1→4)-β-D-glucuronopyranosyl unit of serovar 9. Epitope definition was arrived at through use of the pure, chemically defined glycopeptidolipid antigens and neoglycoproteins containing the chemically synthesized distal sugars of some select serovars. These monoclonal antibodies combined with the already published information on the structure of the antigen determinants and the tools used to arrive at these structures provide powerful means for fundamental studies on the role of these antigens in immunopathogenesis and for the precise mapping of the epidemiology of opportunistic infections caused by *M. avium.*—Authors' Abstract

Snider, D., Jr., Bridbord, K., and Hui, F. Research towards global control and prevention of tuberculosis with an emphasis on vaccine development. Rev. Infect. Dis. **11** Suppl. 2 (1989) S335–S490.

This supplement consists of presentations made at a Fogarty International Center Workshop on control and prevention of tuberculosis held in Bethesda, Maryland, U.S.A., on 3–5 November 1987. The extent of the problem is stressed by D. Snider in the Introduction: over 1 billion of the world's population (5 billion) is estimated to be infected with *Mycobacterium tuberculosis.* and 3 million people are thought to die from tuberculosis each year.

The review-type articles, most with figures, tables, and good reference lists, are arranged in 6 sections. In the section on present approaches to tuberculosis control and prevention, first K. Styblo gives an overview of the global tuberculosis situation concentrating on control in developing countries, then J. H. Grosset discusses the present status of chemotherapy for the disease. The story of the variable efficacy of BCG vaccination against tuberculosis and leprosy is retold by P. E. M. Fine with emphasis on implications for the evaluation of new antimycobacterial vaccines. A. Pio discusses the impact of present control methods (BCG vaccination and case management) and concludes that new developments in technology for diagnosis and control are needed.

W. W. Stead presents the clinical and epidemiological perspectives of the pathogenesis of tuberculosis in the first paper of the second section on the implications of the pathogenesis of tuberculosis for vaccine development. A. M. Dannenberg, Jr., follows with a review of immune mechanisms in

pulmonary tuberculosis. The interactions of HIV infection and tuberculosis and implications for BCG vaccination are reported by T. C. Quinn. D. W. Smith and E. H. Wiegeshaus describe how animal models can help us to understand the pathogenesis of human tuberculosis. Finally in this section, two papers are concerned with the genetics of the immune response to mycobacterial antigens: on the *Bcg* gene in mice (E. Skamene) and the HLA class 2 genes in humans (R. R. P. de Vries).

Four papers make up the section on molecular biology of mycobacteria: on mycobacteriophage vector systems (W. R. Jacobs, *et al.*), a cloned DNA fragment for identification of *M. tuberculosis* (R. J. Patel, *et al.*), the cell wall structure of mycobacteria (P. J. Brennan), antigens of mycobacteria recognized by monoclonal antibodies (D. B. Young and A. Mehlert).

In the section on immunology, W. F. Piessens gives an introduction to the immunology of tuberculosis; J. R. Lamb, *et al.* report on the identification of mycobacterial antigens recognized by T lymphocytes; S. H. E. Kaufmann describes studies *in vitro* on the cellular mechanisms involved in immunity to tuberculosis; and J. J. Ellner and R. S. Wallis discuss immunosuppressive mechanisms resulting from active tuberculosis.

The short section on new developments includes an article by B. R. Bloom on molecular genetic approaches to vaccine development for bacterial pathogens and another by P. Sensi on approaches to the development of new antituberculosis drugs. Finally, "Future directions and priorities" consists of: laboratory techniques for the rapid diagnosis of tuberculosis applicable in developing countries (T. M. Daniel), new experimental drugs for the treatment of tuberculosis (F. Parenti), and problems associated with evaluation of the protective potency of new tuberculosis vaccines (E. H. Wiegeshaus and D. W. Smith).—C. A. Brown (Trop. Dis. Bull.)

Tsukamura, M., Mizuno, S. and Miyama, A. Different correlations of drug susceptibilities to colonial morphology in *Mycobacterium avium* complex strains. Microbiol. Immunol. **33** (1989) 1001–1011.

In *Mycobacterium avium* and *M. intracellulare* complex strains isolated from patients who were not treated previously by any antituberculosis drugs or from fowls, the colonial morphology, smooth, domed, opaque (SmD) or smooth, flat, transparent (SmT) colonial forms, significantly correlated with susceptibilities to rifampin, minocycline, streptomycin, kanamycin, enviomycin, ethambutol, and sulfadimethoxine; whereas it did not correlate with susceptibilities to isoniazid, cycloserine, and ethionamide. Strains with the SmT colonial morphology were more resistant to the former seven drugs than strains with the SmD colonial morphology. Since the susceptibilities to antituberculosis drugs with large molecules correlated with the colonial morphology, it has been suggested that a permeability barrier that allows passage of small molecules but prevents passage of large molecules exists in the strains with the SmT colonial morphology.—Authors' Abstract

van Eden, W., Hogervorst, E. J. M., van der Zee, R., van Embden, J. D. A., Hensen, E. J. and Cohen, I. R. The mycobacterial 65 kD heat-shock protein and autoimmune arthritis. Rheumatol. Int. **9** (1989) 187–191.

Arthritis—induced experimentally in rats by immunization with mycobacteria—has been shown to depend on specific T-cell recognition of an epitope present on the mycobacterial 65-kDa heat-shock protein. This particular epitope has been observed to have a structural mimicry with a cartilage-associated molecule present in the joints. Since the bacterial heat-shock proteins and the cartilage-associated molecules are of a conserved nature, one might infer from the experimental model that in humans similar mimicry could play a role in the initiation of autoimmune arthritis. Recent findings from the analysis of immunological reactivity to the 65-kDa in rheumatoid arthritis patients seem to support such a role for the mycobacterial 65-kDa heat-shock protein in human disease.—Authors' Summary

Verstijnen, C. P. H. J., Schöningh, R., Kuijper, S., Bruins, J., v. Ketel, R. J., Gro-

othuis, D. G. and Kolk, A. H. J. Rapid identification of cultured *Mycobacterium tuberculosis* with a panel of monoclonal antibodies in Western blot and immunofluorescence. Res. Microbiol. **140** (1989) 653–666.

This paper describes the identification of cultured mycobacteria with a panel of monoclonal antibodies directed against species-specific epitopes in a Western blot (WB) test and in an immunofluorescence test (IFT). In WB, we identified mycobacteria of the *Mycobacterium tuberculosis* complex (*M. tuberculosis, M. africanum, M. bovis, M. bovis* BCG, and *M. microti*) with 10^8 bacteria. In the IFT, we identified mycobacteria of the *M. tuberculosis* complex, the *M. avium* complex (*M. avium, M. intracellulare* and *M. scrofulaceum*) and *M. kansasii* with 10^7 bacteria.

Using a panel of 105 mycobacterial patient isolates, we compared identification by WB and IFT with conventional, culture and biochemical identification. Identification of the *M. tuberculosis* complex in WB had a specificity and sensitivity of 96.3% and 98.3%, respectively. Identification in IFT of the *M. tuberculosis* complex had a specificity and sensitivity of 94.6% and 89.7%, respectively. Since the identification of mycobacteria with monoclonal antibodies requires only a small number of bacteria, these tests will reduce by several weeks the time necessary for microbiological identification.—Authors' Summary

Yajko, D. M., Nassos, P. S., Sanders, C. A. and Hadley, W. K. Killing by antimycobacterial agents of AIDS-derived strains of *Mycobacterium avium* complex inside cells of the mouse macrophage cell line J774. Am. Rev. Respir. Dis. **140** (1989) 1198–1203.

The murine macrophage continuous cell line J774 was used to measure the ability of antimicrobial agents, either singly or in combination, to kill intracellular *Mycobacterium avium* complex. All of 14 strains of *M. avium* complex, isolated from patients with AIDS, grew inside J774 cells during an incubation period of 7 days. The susceptibility of macrophage-ingested *M. avium* complex to antimicrobial agents was determined by comparing the number of colony-forming units (cfu) of *M. avium* complex inside untreated macrophages at the time of drug addition with the number of cfu present in macrophages after treatment with drugs for 7 days. Simultaneous experiments were carried out in broth medium without macrophages in order to compare killing of free mycobacteria with killing of macrophage-ingested mycobacteria. Antimicrobial agents (rifampin, rifabutin [Ansamycin], ethambutol, ciprofloxacin, clofazimine, isoniazid, and amikacin) were tested using concentrations that are achievable in the serum of patients. Among drugs known to penetrate macrophages, there was 96.2% agreement in susceptibility test results between the broth experiments and the J774 experiments when single drugs were tested, but only 74% agreement when combinations of drugs were tested. Killing of *M. avium* complex inside J774 cells by any single drug was uncommon. However, killing in J774 cells occurred against 10 of 11 (91%) strains with the combination of rifabutin + ethambutol + ciprofloxacin and against all of seven strains tested with the combination of rifabutin + ethambutol + amikacin. Interpretive criteria of *in vitro* susceptibility data need to be developed so that these interpretations correlate with a predictable clinical response in patients.—Authors' Summary