CURRENT LITERATURE

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

General and Historical

Jacobson, R. The face of leprosy in the United States today. (Editorial) Arch. Dermatol. **126** (1990) 1627–1630.

Obviously we have far to go to conquer this ancient disease, but we have made excellent progress and leprosy has been, to a considerable degree, mainstreamed into U.S. medicine. Such progress is likely to continue.—From the Editorial

Rees, R. J. W., ed. Tuberculosis and leprosy. Br. Med. Bull. **44** (1988) 523–820.

This book contains 20 papers on various aspects of these important mycobacterial diseases written with one exception by U.K.-based workers. The exception is the introduction by Noordeen and Godal which outlines the approach of the World Health Organization special programs channeled through scientific working groups. The next three papers are concerned with the genetics of mycobacteria and the application of molecular biology to their study, the metabolism of mycobacteria, and the structure of mycobacterial antigens, respectively. Then follow five papers discussing the immunological and immunopathological responses to infection with mycobacteria, and highlighting in particular recent studies with T-cell clones, the role of the macrophage in resistance and immunity, and progress in the development of immunodiagnostic assays. The "bench" section of the book finishes with an overview by the editor of the unique contribution of animal models to leprosy research. The second half of the book is concerned with the clinical diseases, how they appear in the community, and how attempts are made to control them by vaccination or chemotherapy. Due prominence is given to case-finding and chemotherapy in control and the possibility of new drugs but this topic is balanced by appropriate consideration of vaccine strategy and the creation of new vaccines by genetic manipulation. There is more on tuberculosis than leprosy. The final chapter in the book discusses the impact of HIV infection/AIDS on the incidence of mycobacterial disease, which impact, given the immunocompromised state found in these patients, should give strength to those looking for new drugs. Each chapter is well referenced and there is a seven-page index.—I. N. Brown (*Trop. Dis. Bull.*)

Chemotherapy

Gelber, R. H. Progress in the chemotherapy of leprosy: status, issues and prospects. Prog. Drug Res. **34** (1990) 421–425.

This is a well-written review of leprosy chemotherapy with 118 references.—RCH

Gelber, R. H., Rea, T. H., Murray, L. P., Siu, P., Tsang, M. and Byrd, S. R. Primary dapsone-resistant Hansen's disease in California; experience with over 100 *Mycobacterium leprae* isolates. Arch. Dermatol. **126** (1990) 1584–1585.

We found that in the years 1978 through 1981 only 1 of 54 previously untreated patients with Hansen's disease was found to harbor dapsone-resistant *Mycobacterium leprae.* That single strain was only partially resistant, i.e., it was resistant to 0.0001% dapsone in a mouse diet but not to higher concentrations. During the years 1983

through 1988, *M. leprae* from 47 previously untreated patients presenting to clinics in San Francisco, California, U.S.A. and Los Angeles, California, U.S.A., grew in mice. None of these strains was found to be dapsone resistant. Thus, from 1978 through 1988 only 1 of 101 *M. leprae* isolates obtained from skin biopsy specimens from patients with leprosy was found to be resistant to dapsone. We have concluded that primary dapsone resistance still does not appear to be a significant problem in California. Owing to the fact that our single resistant case and those reported from international sources are, in general, partially resistant, the potential importance of partial dapsone resistance is discussed.—Authors' Abstract

Girdhar, B. K. Immunopharmacology of drugs used in leprosy reactions. Indian J. Dermatol. Venereol. Leprol. **56** (1990) 354–362.

On the whole, it has been found that the treatment of leprosy hardly has any effect on the reaction or its outcome. Therefore, in order to prevent emergence of resistance, it is advocated that basic antileprosy drugs must be continued even during reactions, unless the latter is life threatening. Although several compounds have been investigated/applied in the management of the two types of reactions in leprosy, only a few are actually available for field application and for treatment of hospitalized patients. Other drugs either give equivocal response, or are too toxic and expensive. The advances in understanding of immunopathology are opening up new horizons for investigations of other immunomodulatory drugs. Although the problem of a lack of precise methods of testing the drugs for their modes of activity exists, many drugs are becoming available for the purpose of ameliorating patient morbidity.—From the Article

Ji, B. and Grosset, J.-H. Recent advances in the chemotherapy of leprosy. (Editorial) Lepr. Rev. **61** (1990) 313–329.

Within the next 5 years, a substantial increase of information about the long-term therapeutic effects of the current MDT regimens will be accumulated, screening and synthesizing of new drugs will be continued and expanded, clinical trials of various new antileprosy drugs and field trials of new combined regimens will be mounted. However, the development of more powerful regimens does not necessarily result in better disease control, the key factor is to apply the effective regimens properly under routine field conditions. There is still a large gap between the number of cases who have been or are being treated with MDT and the total number of registered cases, needless to mention the gap between the former figure and the total number of estimated cases, which is probably still between 10 and 12 million in the world. The gap is particularly wide in Africa, only 19% of the registered cases are under MDT as compared to 50% of cases for the rest of the world, although the problem of leprosy on the African continent is significant. To control leprosy eventually, the gap should be reduced to the minimum. The weakness in the operational aspects are always the reasons that MDT cannot be implemented successfully. It appears that operational research, including health systems research and social-economic research, may provide a better understanding of the reasons for and the possible approaches to cope with the weakness in operational aspects. Unfortunately, this has been a neglected area in leprosy research. Therefore, all efforts should be made to promote operational research. The other important research area is related to leprosy reactions and nerve damage, which may occur during or even after MDT. Within the last two decades, there has been almost no progress either in prevention or in treatment of these two important clinical events. Basic research leading to a better understanding of the mechanisms are needed, and appropriate animal models should be established.—From the Editorial

May, D. G., Porer, J. A., Uetrecht, J. P., Wilkinson, G. R. and Branch, R. A. The contribution of *N*-hydroxylation and acetylation to dapsone pharmacokinetics in normal subjects. Clin. Pharmacol. Ther. **48** (1990) 619–627.

The relative importance of *N*-hydroxylation and acetylation of dapsone to the oral clearance of dapsone (100 mg) was investigated in seven healthy volunteers. Plasma

dapsone and monoacetyldapsone concentrations rose rapidly with subsequent similar monoexponential elimination. The oral clearance of dapsone was low (33 ± 14 ml/min), with a threefold variability. Four subjects were identified as fast acetylators; however, differences in acetylation did not explain the variability in oral clearance. The cumulative urinary recoveries of dapsone and its hydroxylamine were approximately 20% of the dose. The formation clearance of hydroxylamine, which exhibited a tenfold intersubject variability, was closely associated with the oral clearance of dapsone ($r = 0.96$). Thus, the formation of the hydroxylamine is more important than acetylation in determining dapsone's intersubject variability in oral clearance. Variation in *N*-hydroxylation may have clinical consequences, because the hydroxylamine is considered to be important in dapsone-mediated toxicity.—Authors' Abstract

Miao, Z.-H., *et al.* [Histopathological changes in the skin of MB leprosy after MDT.] China Lepr. J. **6** (1990) 141–142. (in Chinese)

The authors report pathologic changes in the skin of 30 multibacillary leprosy patients who have taken MDT for 5 years. They found that the BIG, GF and HI steadily declined during and after treatment. The greatest decrease was evident in the fifth year after treatment. The BIG of 19 cases was negative and the foamy cell granuloma resolved in 11 cases.—Authors' English Abstract

Naik, S. S., Gole, D. H., Neet, M. R. and Dongre, V. V. Pattern of drug compliance in leprosy patients attending urban centres—a longitudinal study. Indian J. Lepr. **62** (1990) 305–309.

The pattern of drug compliance in 485 leprosy patients attending urban leprosy centers in Bombay was studied for 2 years. The study subjects included 113 patients with paucibacillary leprosy under dapsone monotherapy, 241 patients with paucibacillary leprosy under multidrug therapy, and 131 patients with multibacillary leprosy under multidrug therapy. Their urine samples had been checked at least 6 times during the 2 years by the DDS tile test at the time of their clinic attendance. The urine test results were not disclosed to the patients, but patients showing negative results were counseled about the need for regular drug intake. Thirty-five percent of the patients were "regular throughout," 13% were "irregular throughout" and the other 52% who "tended to be irregular" in their drug intake became "regular" after counseling. Regularity in drug compliance was better in patients on multidrug therapy than in those on monotherapy. It is suggested that periodic testing of urine for checking for regularity of drug intake and subsequent counseling of patients should be made a routine practice to maintain drug compliance at a high level.—Authors' Abstract

O'Sullivan, S., Corcoran, M., Byrne, M., McGrath, S. and O'Kennedy, R. Absorption and analysis of clofazimine and its derivatives. Biochem. Soc. Trans. **18** (1990) 346–347.

Clofazimine (B663), a red iminophenazine dye, is very effective in the treatment of leprosy. Its mode of action is thought to be due to its inhibition of the template function of DNA. Resistance to the antileprosy agents rifampin and dapsone has been widely reported, and in 1982 a case of clofazimine-resistant leprosy was described. Hence, a number of analogs of clofazimine were synthesized in order to develop agents that might overcome such resistance. Studies on clofazimine and analogs were performed to determine which compounds had the required absorption characteristics in mice. Animals (8–10-week-old Schofield mice) received a daily dosage of 20 mg day^{-1} kg^{-1} by gavage (0.4 ml) over a period of 3 weeks using a modified 5-cm human spinal needle. After 21 days the mice were killed. The lungs, liver, spleen and pelvic fat were removed and weighed. Groups of three to six mice were used for each drug studied. The amount of drug extracted was then determined spectrophotometrically. These results clearly demonstrate the complex relationship between molecular structure and characteristics, absorption and antileprosy activity.—*From* the article

Shoukrallah, I., Sakla, A. and Wintersteiger, R. Spectrophotometric determination of dapsone by using 9-chloroacridine as a chromogenic reagent. Pharmazie **45** (1990) 675–677.

A spectrophotometric method for the quantitative determination of dapsone has been developed through a condensation reaction of 9-chloroacridine as a chromogen and the amino groups of dapsone. The reaction variables were investigated and optimized. The resultant colored products are stable and were synthesized. The application of the present method to the determination of dapsone in commercial tablets gave satisfactory results and was compared with the official methods. The proposed method is simple, sensitive, reproducible and accurate.—Authors' Abstract

Wele, D. S. Health education for the successful implementation of MDT. Indian J. Lepr. **62** (1990) 346–350.

The importance and goals of health education in leprosy are pointed out. The responsibilities of the health education are outlined. The role of health education in the context of the MDT program is discussed.—Author's Abstract

Wilkins, E. G. L., Hnizdo, E. and Cope, A. Addisonian crisis induced by treatment with rifampicin. Tubercle **70** (1990) 69–73.

This article presents a rare case in a 59-year-old Asian man with spinal tuberculosis and a paravertebral abscess who developed an acute Addisonian crisis 13 days after starting therapy with rifampin, ethambutol and isoniazid. No evidence of adrenal insufficiency was present before therapy. Four previous examples of this phenomenon are noted following rifampin therapy although similar reports have followed treatment with streptomycin and isoniazid. Rifampin is a potent inducer of microsomal enzymes; the mechanisms involved are not clear although increased steroid metabolism may play a role.—K. C. Watson (*Trop. Dis. Bull.*)

Clinical Sciences

Abraham, A., Sharma, V. K. and Kaur, S. Assessment of testicular volume in bacilliferous leprosy; correlation with clinical parameters. Indian J. Lepr. **62** (1990) 310–315.

Testicular involvement in leprosy was studied in 30 multibacillary (BL/LL) patients. Ten (33.3%) gave a past history of type 2 reactions, of whom 9 (30%) gave a history of testicular pain and/or swelling. Decreased libido was a common complaint (63.3%). Gynecomastia was noted in 3 patients (10%) and altered hair pattern in 11 patients (36.7%). Testicular sensation was impaired in 10 (33.3%) patients. Testicular volume was assessed objectively using the Prader orchidometer and found to be reduced in 9 (30%) patients. Reduction in testicular volume correlated with longer duration of disease and a past history of type 2 reaction.—Authors' Abstract

Atkin, S. L., El-Ghobarey, A., Kamel, M., Owen, J. P. and Dick, W. C. Clinical and laboratory studies in patients with leprosy and enthesitis. Ann. Rheum. Dis. **49** (1990) 715–717.

In a combined clinical, radiological, and laboratory study of 77 patients throughout the leprosy spectrum, 10 patients had an enthesitis [inflammation of the site of attachment to bone of tendon, ligament, or joint capsule] which has not been described previously as far as is known and which was not associated with the characteristics of erythema nodosum leprosum reactions. C-reactive protein and α_1 acid glycoprotein values were significantly lowered only in those patients with leprosy and enthesitis. No radiological abnormalities were found.—Authors' Abstract

Bhatia, V. N., Dhandayuthapani, S., Ananthan, D., Rajendran, M., Vasanth, B., Jayasingh, K. and Vinod Kumar, C. H. D. Sub-clinical infection with *Mycobacterium leprae* in household contacts of

leprosy. Indian J. Lepr. **62** (1990) 296–304.

Eight-hundred-seventy household contacts of leprosy patients were examined for subclinical infection with *Mycobacterium leprae* by smear (skin and nasal), lepromin and FLA-ABS tests: 0.6%, 3.3%, 71.5% and 14.4% of the contacts were found to be positive for skin smear, nasal smear, lepromin and FLA-ABS tests, respectively. An analysis of the results revealed that 4% of the lepromin-positive contacts and 3.6% of the lepromin-negative contacts were positive to both FLA-ABS and skin or nasal smear.—Authors' Abstract

Carrazana Hernández, G. B., Ferrá Torres, T. M. and Perez, R. P. [A study of disabilities caused by leprosy.] Rev. Leprol. Fontilles **17** (1990) 547–555. (in Spanish)

In order to determine the degrees of disability caused by leprosy, a total of 137 patients with Hansen's disease were examined in three health areas of Camaguey City, Cuba, during 1989. Incidence rates from 1987 to 1989 were excluded. The percentage of cases with disabilities was 21.2 (29 patients). There was a preponderance of lepromatous leprosy (96.6%); 58.6% of patients with disabilities had had the disease for 20 or more years. Males (62.0%) and the group aged 40 and more years predominated. Second-degree disabilities were the most relevant on hands, feet and eyes. Trophic manifestations on the hands most commonly encountered were: preacher hand (20.7%), severe mutilation (17.3%), and claw hand (13.8%). Insensitivity (20.7%), mal perforans (20.7%), and severe mutilation were significant on the feet. Blurred vision (27.6%) was common regarding the eyes.—Authors' English Summary

Castaño Hernández, S. T. and Carrazana Hernández, G. B. [Changes in carbohydrate metabolism in lepromatous leprosy.] Rev. Leprol. Fontilles **17** (1990) 565–573. (in Spanish)

Carbohydrate metabolism (CM) in 84 lepromatous leprosy patients was studied in the health areas of Camaguey City, Cuba, at the beginning of 1989. In order to avoid interference in the results of the oral glucose tolerance test (GTT), cases suffering acute diseases, erythema nodosum leprosum, and those who were orally treated with drugs were excluded from this study. All patients were submitted to GTT according to the National Norms for Endocrinology. Corporal Mass Index was employed for the diagnosis of obesity. The results obtained showed CM disorders in 7.1% of lepromatous leprosy patients; there was a preponderance of the cases with altered GTT in relation to clinical diabetics; males (83.3%) and the group aged 56–75 years predominated; most of the cases with CM disorders were not obese; 66.7% of the patients had no familial history of diabetes mellitus and 100.0% of them presented only dermatoses.—Authors' English Summary

Chanteau, S., Cartel, J.-L., Perani, E., N'Deli, L., Roux, J. and Grosset, J.-H. Relationships between PGL-I antigen in serum, tissue and viability of *Mycobacterium leprae* as determined by mouse footpad assay in multibacillary patients during short-term clinical trial. Lepr. Rev. **61** (1990) 330–340.

In connection with a 56-day controlled clinical trial for comparing the therapeutic effects between pefloxacin and ofloxacin in 21 lepromatous patients, we have studied the relationships between PGL-I antigen level in serum and in skin and serum PGL-I antibody titer on the one hand, and the viability of *Mycobacterium leprae,* as measured by serial mouse foot pad inoculations, and other bactericidal parameters on the other. Before and during treatment, significant correlation was found between serum PGL-I level and the morphological index (MI), and with the number of viable organisms per mg skin tissue. However, neither serum PGL-I antibody titer nor skin PGL-I antigen level showed significant change during the 56-day trial. Because the reduction of serum PGL-I level was well correlated but less pronounced as compared with the evolution of viable organisms during treatment, the serum PGL-I antigen assay may be useful as an early indicator of response to chemotherapy in short-term clinical trial, but it is unlikely to replace mouse foot pad inoculation for the evaluation of viability of *M. leprae.*—Authors' Summary

Gupta, A. B., Tutakne, M. A. and Haldar, B. Measurement of some biophysical parameters in skin lesions of leprosy. Indian J. Dermatol. Venereol. Leprol. **56** (1990) 367–370.

Transepidermal water loss (TEWL), high frequency electrical conductance (HFC), and the hydration state index (HSI) were measured in skin lesions of 30 paucibacillary leprosy patients and compared with the contralateral uninvolved skin. While the TEWL, HFC and HSI all showed lower values in the lesion site, as compared to the contralateral skin sites, the differences between the two sets of values were significant in HFC and HSI only at 2% and 1% level, respectively. A significant positive correlation ($r = 0.69$) was found to exist between these two parameters. The parameters correlate well with the known reduced sweating in skin lesions of TT and BT leprosy and may therefore be considered as good objective parameters to confirm hypohydrosis in suspected skin lesions of leprosy.—Authors' Abstract

Gupte, M. D., Valli Shayee, R. S., Nagaraju, B., Ramalingam, A., Lourdusamy, G. and Kannan, S. Inter-observer agreement and clinical diagnosis of leprosy for prophylaxis studies. Indian J. Lepr. **62** (1990) 281–295.

Classical diagnosis is still the most useful tool for detecting early cases of leprosy in field research. In prophylaxis studies accuracy of the clinical diagnosis of leprosy is important during intake as well as for measuring efficacy of the intervention. This paper reports our observations regarding the extent of inter-observer variations in the clinical diagnosis of leprosy and its implications for a prophylaxis study. Information on 225 suspects and cases of leprosy, each examined independently by three senior workers after initial standardization, was used for this purpose. Agreement among the examiners regarding the presence of skin patch, thickened nerve trunk, and sensory deficit was fairly high (Kappa = 0.7). Agreement on the presence of infiltration in a skin patch was not satisfactory (Kappa = 0.4–0.5). It was observed that in the clinical diagnosis of leprosy, presence of skin patch and sensory deficit, as well as thickened nerve trunk and related anesthesia were correlated observations. The influence of inter-observer variations on defining the leprosy problem in the community can be quite large. The paper suggests some ways of overcoming the problem.—Authors' Abstract

Harle, J.-R., Disdier, P., Kaplanski, G., Tamalet, C., Weiller-Merli, C., Bonerandi, J.-J. and Weiller, P.-J. Lepromatous leprosy and seropositivity for HTLV-I. (Letter) Am. J. Med. **89** (1990) 535.

Leprosy and HTLV-I (human T-cell lymphotrophic virus type I) infection are both endemic conditions in the Caribbean. It is surprising that their association has never been mentioned. We report what we believe to be the first such case.—From the Letter

Jadhav, V. H., Patki, A. H. and Mehta, J. M. Thalidomide in type-2 lepra reaction—a clinical experience. Indian J. Lepr. **62** (1990) 316–320.

A clinical experience of using thalidomide in type-2 lepra reaction (ENL) in 90 male patients—57 with lepromatous leprosy (LL) and 33 with borderline lepromatous leprosy (BL)—is described. All the patients responded well although some took a longer time to improve. No major side effects were observed except for giddiness in 10 and gastrointestinal upsets in 7 patients. Thalidomide thus appears to be a very effective drug in the treatment of severe type-2 lepra reaction and apart from its historically well-documented embryopathic effects, does not seem to have any other serious side effects in the patients under study.—Authors' Abstract

Jenkins, D., Papp, K., Jakubovic, H. R. and Shiffman, N. Leprotic involvement of peripheral nerves in the absence of skin lesions; case report and literature review. J. Am. Acad. Dermatol. **23** (1990) 1023–1026.

In the absence of clinically apparent cutaneous lesions, primarily neural leprosy is uncommon. Primarily neural leprosy presents clinically as a peripheral neuropathy

that most frequently affects motor nerves and that occasionally involves sensory nerves as well. The long incubation period for leprosy and its occurrence outside endemic areas often lead to delayed diagnosis. We present a case of glove and stocking hypoesthesia, weakness of the flexor muscle of the right great toe, palpable thickening of the right popliteal nerve, and hypoesthetic but normal-appearing areas on the back, which developed in a Trinidadian immigrant who lived in Canada for 16 years. A skin-biopsy specimen obtained from a visibly normal but hypoesthetic area on the back demonstrated a few acid-fast bacteria in small dermal nerves, in arrector pili smooth muscle, and in rare perivascular histiocytes, associated with a sparse mixed inflammatory cell infiltrate. The patient responded well to therapy with dapsone, rifampin, and clofazamine. A classification and review of primarily neural leprosy is presented. Our patient represents the first reported case of primarily neural borderline lepromatous leprosy in Canada.—Authors' Abstract

Leonard, G., Sangare, A., Verdier, M., Sassou-Guesseau, E., Petit, G., Milan, J., M'Boup, S., Rey, J.-L., Dumas, J.-L., Hugon, J., N'Gaporo, I. and Denis, F. Prevalence of HIV infection among patients with leprosy in African countries and Yemen. J. Acquir. Immune Defic. Syndr. **3** (1990) 1109–1113.

Screening for human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) antibodies was carried out in the serum of 1245 leprous patients and 5731 controls selected in nine different centers from the Congo, Ivory Coast, Senegal, and Yemen Arab Republic. In Yemen, all sera were negative. In the Congo, the seropositivity among patients and controls was, respectively, 3.8% and 5.2%; in Senegal, it was 1.3% and 0.6%; and in the Ivory Coast, 4.8% and 3.9%. Differences were not statistically significant, even considering lepromatous or tuberculoid forms (3.6% and 3.7%, respectively). HIV-2 antibodies were only detected in subjects from the Ivory Coast and Senegal. Using appropriate criteria for seropositivity (confirmation by Western blot, reactivity to HIV envelope glycoproteins) and a large selection of patients (several countries with several centers), it appears that leprosy (and especially the lepromatous form) is not a factor for HIV infection.—Authors' Summary

Luo, Y.-R., *et al.* [A survey of eye lesions in leprosy.] China Lepr. J. **6** (1990) 131–135. (in Chinese)

Sixty-five leprosy inpatients with 30 cures in two leprosaria were ophthalmologically examined in 1987. It was found that the rate of eye diseases is 87.7%, of which most is in the cornea and the next is in the palpebrae and uvea. The vision was normal in 47 eyes, abnormal in 83, and low or blind in 37 eyes. Pathogenesis and treatment of leprosy lesions in the eye are discussed. The authors point out that the curability rates of blindness and low vision are 66.7% and 100%, respectively, and emphasize the importance of early detection and early treatment of eye diseases in leprosy.—Authors' English Abstract

Saxena, U., Ramesh, V., Misra, R. S. and Mukherjee, A. Giant nerve abscesses in leprosy. Clin. Exp. Dermatol. **15** (1990) 349–351.

Two leprosy patients with neuritis caused by giant abscesses involving almost the entire ulnar nerve are described. One patient, who also had skin lesions, was diagnosed histopathologically as having borderline tuberculoid leprosy both on skin and nerve biopsy, and the other, with only nerve involvement, belonged to the pure neuritic group. The lepromin test was strongly positive (with a vesicular reaction in one patient) and lymphocyte transformation to *Mycobacterium leprae* antigen was raised. These lesions can be easily mistaken for a peripheral nerve tumor in places were leprosy is uncommon. A brief account of the management of nerve abscess in leprosy is given.—Authors' Summary

Sehgal, V. N., Joginder, Sharma, V. K. and Prakash, S. K. Cell-mediated and humoral immunity in leprosy in children. J. Dermatol. **17** (1990) 356–361.

Cell-mediated and humoral immunity were studied in 25 children between 0 and

14 years with leprosy. Cell-mediated immunity was studied *in vivo* by lepromin and epicutaneous sensitization with dinitrochlorobenzene (DNCB) and T lymphocytes and their subpopulations (T4 and T8) in the peripheral blood. Humoral immunity was evaluated by B-lymphocyte count and immunoglobulins (IgG, IgM and IgA). Along with complement, component C3 was also measured in the serum. Lepromin (Mitsuda) and DNCB responses were significantly poor in mid-borderline (BB) leprosy. The hematological profile, including T lymphocytes, their subpopulations, B lymphocytes, serum immunoglobulins, and C3, were found to be normal in all forms of leprosy. The relatively short duration of disease and the low bacterial load may explain these findings.—Authors' Abstract

Sen, R., Chaudhary, S. D., Dixit, V. B. and Jain, V. K. Bone marrow cyto-morphological changes in multibacillary leprosy. Indian J. Lepr. **62** (1990) 321–327.

Seventy-two cases of multibacillary leprosy were investigated for cytomorphological changes and the presence of lepra bacilli in bone marrow. These patients were divided in two groups. Group A (28) consisted of new cases and group B (44) of those receiving treatment. Myeloid hyperplasia was mostly seen in patients of group B who had erythema nodosum leprosum. Megaloblastic change in erythroblasts was seen frequently in both the groups. While the average number of plasma cells and macrophages was on the higher side of normal range, detection of a large number of plasma cells underlined enhanced humoral response and created a diagnostic problem with multiple myeloma. Morphological changes in the macrophages, their collections and epithelioid cell granulomas were observed in bone marrow. Their nature and significance is discussed.—Authors' Abstract

Sreevatsa, Malaviya, G. N., Husain, S., Girdhar, A., Bhat, H. R. and Girdhar, B. K. Preliminary observations on myiasis in leprosy patient. Lepr. Rev. **61** (1990) 375–378.

Out of 3350 leprosy patients attending the surgical outpatient department for various ulcerative lesions, 18 patients had typical symptoms of myiasis. Maggots were collected in 5 cases from the nose, in 3 cases from ulcers of the hand, and in 10 cases from ulcers of the foot. It was possible to rear the maggots into flies in 8 out of 18 cases. The flies were identified as *Sarcophaga ruficornis* and *Chrysomyia bezziana*.—Authors' Summary

Immuno-Pathology

Anderson, D. C., Van Schooten, W. C. A., Barry, M. E., Janson, A. A. M. and de Vries, R. R. P. Use of flanking sequences to study secondary structure-activity correlations of a *Mycobacterium leprae* T cell epitope. Eur. J. Immunol. **20** (1990) 2691–2697.

The 65-kDa protein of the intracellular pathogen *Mycobacterium leprae* is prominent in the immune response to this mycobacterium, and individual T-cell epitopes from this protein sequence have been defined. We have tested the stimulatory activity of extended analogs of the minimal peptide representing one such epitope, LQAAPALDKL, with a variety of tetrapeptide extensions added to enhance or destabilize α helix formation. The conformational potential of the peptides was measured by circular dichroism using aqueous trifluoroethanol as a secondary structure inducer. Although analogs with high helical potential activated T cells at low concentrations, a less helical variant was similarly potent. Activity also did not correlate with predicted overall α helical amphipathicity. One analog was found which stimulated T-cell proliferation in the 50 pM range. The effect of tetrapeptide extensions on epitope activity is not consistent with the importance in activity of only a single

stable secondary structure such as an α helix.—Authors' Abstract

Arnoldi, J., Gerdes, J. and Flad, H.-D. Immunohistologic assessment of cytokine production of infiltrating cells in various forms of leprosy. Am. J. Pathol. **137** (1990) 749–753.

The aim of this study was to determine cytokines in human leprosy lesions by means of immunohistologic examination. Cryostat sections of skin biopsies from 57 patients with various forms of leprosy were immunostained according to the APAAP method, using monoclonal antibodies against interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-alpha), interferon-gamma (IFN-gamma), and, in addition, against CD 1 antigen. Granulomas in biopsies of untreated patients with tuberculoid leprosy showed large amounts of cells positive for IL-1β, TNF-alpha, IFN-gamma, and CD 1; whereas no positive signals could be detected in untreated patients with lepromatous leprosy. However, in those biopsies obtained from lepromatous leprosy patients undergoing chemotherapy, positive staining for cytokines as well as subepidermal Langerhans cells increased to a detectable amount. Remarkably, in tuberculoid leprosy patients, the number of IL-1β-positive cells did not vary under therapy, while the number of TNF-alpha and IFN-gamma reactive cells decreased. These results suggest that immunohistologic determination of cytokines in combination with the assessment of subepidermal Langerhans cells in human leprosy lesions may be used as a parameter for the patient's status of cell-mediated immunity under chemotherapeutic treatment.—Authors' Abstract

Bottasso, O., Poli, H., Amerio, N. and Merini, J. C. [Influence of treatment with *Mycobacterium leprae* on the immune response in rats; its evaluation in a model of experimental arthritis.] Acta Physiol. Pharmacol. Latinoam. **40** (1990) 19–29. (in Spanish)

Adult male rats "m" strain were treated with heat-killed *Mycobacterium leprae* (Ml) 4×10^7 bacilli/ml, obtained from human lepromas, according to the following scheme: one intraperitoneal -ip- (a) or subcutaneous -sc- (b) injection of 2 ml Ml 2 days before arthritis induction; 1 ip (c) or sc (d) injection of 0.5 ml Ml twice a week during 15 days totaling four injections before induction. Adjuvant arthritis (AA) was elicited 48 hr after injecting 0.1 ml of complete Freund adjuvant in the right hind foot pad. At day 15 of induction, a significant reduction in the severity of arthritis was observed in rats treated with 4 ip injections of 0.5 ml Ml, when compared with the controls. A similar depressive effect on AA was obtained in syngeneic recipient rats when they were transferred with 1×10^8 spleen cells from rats which had been treated with the same protocol. Subsequently, two additional groups of rats, 48 hr before arthritis induction received: a single ip injection of 1×10^8 whole spleen cells or 7×10^7 nonadherent spleen cells from similarly Ml-treated rats. A remarkable reduction of AA was also observed by transferring nonadherent spleen cells. In a latest experiment, this cellular population was unable to reduce the arthritic response in recipient rats when the cells were transferred 48 hr after arthritis induction. On the basis of our results, we conclude that a reiterated exposure to Ml might induce suppressor cells with regulatory activity on AA operating in the preinductive phase of response.—Authors' English Summary

Carrazana Hernandez, G. and Ferrá Torres, T. M. [ELISA results in the prevalence of leprosy after monotherapy.] Rev. Leprol. Fontilles **17** (1990) 449–457. (in Spanish)

ELISA results are presented and analyzed in 328 patients (89.6%) of leprosy prevalence during 1988 in Camagüey City, Cuba, after mono-drug therapy. Specific antigen to *Mycobacterium leprae*—phenolic-glycolipid-I—was used for ELISA. According to the time of treatment ELISA results were correlated with the sanitary classification and clinical forms of leprosy by the Madrid classification. The seronegativity was 77.2% in multibacillary cases; in this group the patients who had been treated for or more than 10 years showed high percentage (46.3%). The seropositivity in paucibacillary cases (15.0%) was seen during the first 5 years of therapy; 54.8% of the lepromatous patients

treated for or more than 10 years were seronegative, but seropositivity persisted in 12.2% of the cases. Borderline, tuberculoid, and indeterminate patients had low seropositivity percentages (12.1; 13.4 and 14.3, respectively) especially during the first 5 years of therapy.—Authors' English Summary

Chanteau, S., Cartel, J.-L., Spiegel, A., Plichart, R. and Roux, J. [Detection of IgM anti-PGL-I for the serodiagnosis of leprosy and for the follow up of contact population in Polynesia. A 5 year situation.] Bull. Soc. Pathol. Exot. Filiales **83** (1990) 649–657.

This study was conducted to assess the usefulness of IgM anti-PGL-I antibody test for the diagnosis of leprosy and of the subclinical infection among contact population. Even if the specificity (97.7%), sensitivity (98% for the multibacillary, but 36% for the paucibacillary) and efficiency (97.7%) of the test for the diagnosis of patients are good, its positive predictive value which is the proportion of true patients among the seropositive subjects detected in the population was very low (1.17% in Polynesia), because of the very low prevalence of leprosy. For the diagnosis of the subclinical infection, our experience based on the follow up of a population of 1123 contacts, showed that such a control program would have low feasibility and low cost-effectiveness. After 5 years, the proportion of individuals developing the disease was not different among the seropositive than among the seronegative subjects. Finally, only 17.5% of the new cases of leprosy detected issued from the contact population followed. In conclusion and in a practical point of view, although this test may contribute to the diagnosis of patients, it is likely useful neither for the diagnosis nor for the prognosis of leprosy in a population.—Authors' English Summary

Chen, J.-L., *et al.* [Preliminary study of delayed hypersensitivity to leprosy antigen induced by killed leprosy bacilli plus BCG.] China Lepr. J. **6** (1990) 135–141. (in Chinese)

After intracutaneous injection of a (heat-killed) vaccine of leprosy bacilli plus BCG in 30 cases of multibacillary (MB) leprosy, localized skin reactions, including redness, induration and ulceration, were found without influence on its usefulness. Repeated injection did not strengthen the reactions. Only after the fifth injection was a general reaction found in 43%, which included iritis and complaints of numbness in the fingers and toes, which might decrease its usefulness. Repeated injection seems be able to induce iritis, but the association between the injection and finger or toe numbness is still uncertain. The injection did not induce type 1 or 2 lepra reaction, but it made the soluble *M. leprae* antigen (SMLA) positive in most cases. The reduced SMLA positivity is unstable. After the seventh injection, the positive rate of SMLA-I (Rees antigen) was 45% and that of SMLA-II (Convit antigen), 35%. After the eighth injection the Mitsuda reaction became positive in 66.7% of the cases, but it still needs following up as to whether the positivity will be sustained or not. SMLA-I and SMLA-II reactions are correlative but their degree is only medium. The positive rate of SMLA-II after repeated injection is higher than that of SMLA-I. There was no correlation between the Fernandez or Mitsuda reaction and the SMLA reaction. The repeated injection did not influence the level of PGL-I in MB cases with weak positivity of PGL-I and negative skin smears.—Authors' English Abstract

Cisse, M. F., Aguis, G., Dindinaud, G., Vaillant, V., Hane, A. A., Castel, O., Grollier, G. and Castets, M. [Evaluation of an ELISA using A60 antigen from *Mycobacterium bovis* BCG: specific IgG and IgM during mycobacterial infections.] Ann. Biol. Clin. **48** (1990) 369–372. (in French)

The authors have evaluated an ELISA (A60-Tb, Anda Biologicals) allowing the detection of specific IgG and IgM against A60 antigen from *Mycobacterium bovis* BCG during mycobacterial infections. This study included sera from 110 African subjects and from 71 French subjects distributed in four clinical groups: 55 tuberculous patients (I); 41 leprous patients (II); 33 pneumonathies (III); and 52 healthy subjects (IV). Serological results were compared taking as reference for the diagnosis of tuberculosis the

positivity of culture and/or that of a direct examination, and for leprosy the positivity of a direct examination associated either with a Mitsuda reaction or with a histopathological examination. IgG was found to be more discriminative than IgM. Considering together the results of groups I and II, the authors found a sensitivity of 95.8% and a specificity of 75.3% with a threshold of 200 U/ml for specific IgG. Anti-A60 antigen antibodies obtained for groups I and II were significantly higher (IgG: $p < 0.0001$; IgM: $p < 0.001$) than those observed in other groups. African subjects presented IgG titers higher than those obtained by French subjects ($p < 0.0001$). IgM response was more frequent among group II (97.6%) than group I (21.8%). However, IgG (26.9%) and IgM titers (30.8%) were detected among group IV. This test would allow a control of therapeutic efficacy with an additional interest for classifying borderline forms of leprosy.—Authors's English Summary

Douglas, J. T., Hirsch, D. S., Fajardo, T. T., Guido, L. S. and Klatser, P. R. Serological monitoring of previously treated lepromatous patients during a course of multiple immunotherapy treatments with heat-killed *Mycobacterium leprae* and BCG. Clin. Exp. Immunol. **82** (1990) 567–573.

Two-hundred-seventy lepromatous patients who had completed treatment received multiple treatments with heat-killed *Mycobacterium leprae* and BCG and were monitored for changes in humoral responses to *M. leprae*-specific antigens. These patients were divided into four treatment groups: placebo (N = 69); BCG (N = 68); *M. leprae* only (N = 71); and BCG + *M. leprae* (N = 62). They were monitored for 15 months, receiving five inoculations for each treatment regimen. Two ELISA systems, one measuring antibodies to *M. leprae*-specific epitopes of the phenolic glycolipid-1 (NDO-ELISA) and the other of 36-kDa protein antigens (INH-ELISA) were used to measure serological changes during this period of immunotherapy. We found no significant increase in serological reactivity with the different treatments, as measured by NDO-ELISA. INH-ELISA similarly showed no significant changes, with the exception of increased values in a small group 13% (36/270) which became skin-test-positive during the course of the study. The NDO-ELISA results indicate that use of heat-killed *M. leprae* or BCG + heat-killed *M. leprae* did not stimulate the humoral response to the semi-synthetic PGL-I antigens of *M. leprae*. Thus, the NDO-ELISA may be useful in monitoring the outcome of vaccine trials in which killed *M. leprae* or *M. leprae* fractions are used, since seroconversion may indicate disease, rather than a response to the vaccine material.—Authors' Summary

Fournie, J.-J., Mullins, R. J. and Basten, A. Isolation and structural characteristics of a monoclonal antibody-defined cross-reactive phospholipid antigen from *Mycobacterium tuberculosis* and *Mycobacterium leprae*. J. Biol. Chem. **266** (1991) 1211–1219.

A low molecular weight antigen of *Mycobacterium leprae* and other mycobacteria was previously defined in our laboratory by means of IgG_{2a} monoclonal antibody termed L4. The antigen had an apparent molecular mass of 4.5–6 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and was assumed to be a glycoprotein on the basis of its staining with periodic acid Schiff and sensitivity to periodate treatment. In the present work, the crossreactive and phospholipidic nature of the antigen, present in *M. tuberculosis*, as well as in *M. leprae* sonicates, was demonstrated and this enabled us to undertake its purification from crude *M. tuberculosis* phospholipidic extracts. The L4-reactive antigen from *M. tuberculosis*, called L4-PIM, was purified by means of silicic acid high-pressure liquid chromatography. Its characterization by gas chromatography and FAB-MS showed the antigen to be the common mycobacterial dimannosylated phosphatidylinositol (PIM_2), the structure of which had been previously established by others. Delineation of the L4 epitope on *M. tuberculosis* L4-PIM revealed the involvement of the *axial* 2-hydroxyl of the α-D-mannosyl residues, without any detectable contribution from the *myo*-inositol. Consequently, L4 was shown to react with PIM_5, the structure of which contains twice the number of epitopes as does PIM_2.

By using both immunostained thin-layer chromatography and indirect enzyme-linked immunosorbent assay, similar L4-PIM epitopes were demonstrated in *M. leprae* sonicate, thereby explaining the crossreactive nature of the L4-monoclonal antibody. Antibodies of IgG class directed against *M. tuberculosis* L4-PIM were detectable in sera from patients with leprosy, but no evidence of T-cell reactivity to L4-PIM was obtained. The demonstration of a correlation of anti-L4-PIM IgG and anti-disaccharide-conjugated bovine serum albumin IgM antibody titers in the sera of leprosy patients indicates that measurement of antibodies directed against L4-PIM may have the potential to be used as a complementary assay to the disaccharide-conjugated bovine serum albumin test for diagnosis and monitoring of patients undergoing leprosy therapy.—Authors' Abstract

Ganju, L., Mukherjee, R., Patra, H. V. and Talwar, G. P. Immuno-Blot analysis of antigens of *Mycobacterium w*: a candidate anti-leprosy vaccine using monoclonal antibodies and patient sera. Zentralbl. Bakteriol. **273** (1990) 378–385.

The presence of determinants immunologically crossreactive with *Mycobacterium leprae* and *M. tuberculosis* in *Mycobacterium w (M. w)* has been revealed by immunoblotting using crossreactive and specific monoclonal antibodies (Moabs) to *M. leprae* and *M. tuberculosis.* Three of the seven *M. leprae* and one of the two *M. tuberculosis* "specific" Moabs showed reactivity with *M. w* antigens. Reactions were also manifest with crossreactive Moabs. One out of three Moabs raised to *M. leprae* and three of six to *M. tuberculosis* demonstrated reactivity with *M. w* antigens. Extensive reactivity of *M. w* antigens was also observed with patient sera; sera from leprosy patients reacted prominently with *M. w* antigens at 14–17 kDa and sera of active tuberculosis patients exhibited reactivity at 21-kDa antigens of *M. w.* Four out of 30 healthy individuals living in an endemic area showed reactivity with *M. w* antigens.—Authors' Abstract

Gonzalez-Abreu, E., Olivares, R., Mora, N. and Gonzalez, A. B. [Monitoring treatment effectiveness in patients with leprosy using solid-phase immunoenzyme assay.] Rev. Cubana Med. Trop. **41** (1989) 333–340. (in Spanish)

Serum samples from 184 leprosy patients are studied by means of solid phase ELISA with the semisynthetic antigen disaccharide-bovine albumin analogous of phenolic glycolipid-I of *Mycobacterium leprae.* Patients are grouped according to the clinical presentation of the disease and course of time elapsed since the institution of therapy. Bacilloscopic examinations were made also in 116 patients who were positive at diagnosis. For serologic testing, absorbance values over 0.160 were considered positive. Results in multibacillary patients showed a gradual and significant decrease both of mean absorbance values and in the seropositive ratio in connection with the length of treatment. It was also found that phenolic antiglycolipid antibodies increased with the value of the bacteriologic index. This system is useful in monitoring the effectiveness of chemotherapy in multibacillary leprosy.—Author's English Summary

Haanen, J. B. A. G., Ottenhoff, T. H. M., Lai A Fat, R. F. M., Soebono, H., Spits, H. and de Vries, R. R. P. *Mycobacterium leprae*-specific T cells from a tuberculoid leprosy patient suppress HLA-DR3-restricted T cell responses to an immunodominant epitope on 65-kDa hsp of mycobacteria. J. Immunol. **145** (1990) 3898–3904.

The polar tuberculoid type (TT) of leprosy, characterized by high T-cell reactivity to *Mycobacterium leprae,* is associated with HLA-DR3. Surprisingly, DR3-restricted low T-cell responsiveness to *M. leprae* was found in HLA-DR3-positive TT leprosy patients. This low responsiveness was specifically induced by *M. leprae* but not by *M. tuberculosis* and was seen only in patients and not in healthy controls. We studied this patient-specific, *M. leprae*-induced, DR3-restricted low T-cell responsiveness in depth in one representative HLA-DR3-positive TT leprosy patient by using T-cell clones. From this patient two types of T-cell clones were obtained: one type was crossreactive with *M. tuberculosis* and recognized an immunodominant epitope (amino acids 3 to

13) on the 65-kDa heat shock protein (hsp), the other type was *M. leprae* specific and reacted to a protein other than the 65-kDa one. To examine whether these *M. leprae*-specific T-cell clones were responsible for the DR3-restricted low responsiveness to *M. leprae,* we tested them for the ability to suppress the proliferation of the DR3-restricted, 65-kDa, hsp-reactive clones. The DR3-restricted, *M. leprae*-specific T cells completely suppressed the proliferative responses of DR3-restricted, crossreactive T-cell clones to the 65-kDa hsp from the same patient as well as from other individuals. Also, DR3-restricted responses to an irrelevant antigen were suppressed by the *M. leprae*-specific T-cell clones. However, no suppression of non-DR3-restricted T-cell responses was seen. Although the mechanism must still be elucidated, this *M. leprae*-induced, DR3-restricted immunosuppression may at least partly explain the observed DR3-associated low T-cell responsiveness in TT leprosy patients.—Authors' Abstract

Lazaro-Medina, A., Tianco, E. A. and Avila, J. M. Additional markers for the type I reactional states of borderline leprosy. Am. J. Dermatopathol. **12** (1990) 417–421.

The histological course of reaction in borderline leprosy has been described by Ridley and Radia. They are dermal edema, dilatation of the lymphatics, swelling of the granulomas, changes in the concentration and distribution of lymphocytes and giant cells, maturity of the histiocytes, and presence of neutrophils. New markers for the condition are spongiosis of the epidermal and follicular epithelium with exocytosis of mononuclear cells, parakeratosis, focal interface changes with occasional individual cell necrosis of keratinocytes, and lastly, follicular mucinosis. Recognition of this reaction is vital in the prevention of deformities secondary to nerve damage.—Authors' Abstract

Li, F.-T., *et al.* [Detection of serum antibody against lipoarabinomannan-B from H37Ra.] Chin. J. Tuberc. Respir. Dis. **13** (1990) 6–8. (in Chinese) English Abstract pp. 62–63.

The serum antibody to lipoarabinomannan-B(LAM-B) purified from *Mycobacterium tuberculosis* (H37Ra) was tested by ELISA in 250 sera, including sera from patients as follows: tuberculosis 96, tubercular pleurisy 11, renal tuberculosis 2, bone and joint tuberculosis 33, tubercular meningitis 16, pulmonary cancer 22, leprosy 20, and normal subjects 50. The positive rate of pulmonary tuberculosis is 69.8%, which is of a similar extent in sera from patients with tuberculosis of miscellaneous organs to be tested except tubercular meningitis, in which only a 18.8% positive rate was observed, indicating the blockage of antibody releasing from pathologic foci into blood stream by blood-brain barrier. The positive rates of leprosy and normal subjects are 50.0% and 2.0%, respectively. No antibody was found among 22 patients with pulmonary cancer.

It is suggested that the existence of an active tubercular lesion in the host might be the basic prerequisite for a positive LAM-B antibody detection. Although LAM-B is a common antigen of both *M. tuberculosis* and *M. leprae,* the low prevalence of leprosy in China makes little influence of the practicability of using this ELISA in epidemiological study and in clinics as an adjutant tool for tuberculosis diagnosis and differential diagnosis.—Authors' English Abstract

Li, S. G., Ottenhoff, T. H M., Van den Elsen, P., Koning, F., Zhang, L., Mak, T. and de Vries, R. R. P. Human suppressor T cell clones lack CD28. Eur. J. Immunol. **20** (1990) 1281–1288.

Previously we showed that certain T-cell lines and clones from a lepromatous leprosy patient displayed a dose-dependent suppression of the proliferation of autologous T cells to *Mycobacterium leprae* but not mitogen or an unrelated antigen. The latter cells were also cloned and did not display this suppressive activity, were CD4+ and proliferated vigorously to *M. leprae* presented by autologous HLA-DR molecules. We shall refer to these cells as T helper (T_h) cells. Most of the suppressive T-cell clones (T_s) were also CD4+ and also proliferated to *M. leprae* presented by HLA-DR, but much less strongly than T_h cells. In this study

we report on our search for a) the mechanism of this apparently antigen-specific suppression by T cells, and b) a possible phenotypic difference between T_h and T_s clones. The two main conclusions are that T_s clones possess a lytic machinery, but that *M. leprae*-specific suppression and cytotoxicity can be clearly dissociated, and that the only phenotypic difference between T_h and T_s is the presence of the CD28 marker on T_h and its absence on T_s clones.—Authors' Abstract

Lyons, J., Sinos, C., Destree, A., Caiazzo, T., Havican, K., McKenzie, S., Panicall, D. and Mahr, A. Expression of *Mycobacterium tuberculosis* and *Mycobacterium leprae* proteins by vaccinia virus. Infect. Immun. **58** (1990) 4089–4098.

Eight *Mycobacterium tuberculosis* and *M. leprae* genes were inserted into the vaccinia virus genome by *in vivo* recombination. The resulting virus recombinants were shown to express five different *M. tuberculosis* proteins (71, 65, 35, 19, and 12 kDa) and three *M. leprae* proteins (65 and 18 kDa and a biotin-binding protein) by Western immunoblot analysis, radioimmunoprecipitation, or black-plaque assay. When injected into BALB/c mice, the recombinants expressing the *M. tuberculosis* 71-, 65-, or 35-kDa protein and the *M. leprae* 65-kDa protein or the biotin-binding protein elicited antibodies against the appropriate *M. tuberculosis* or *M. leprae* protein. These vaccinia virus recombinants are being tested for the ability to elicit immune protection against *M. tuberculosis* or *M. leprae* challenge in animal model systems. The recombinants are also useful in generating target cells for assays aimed at elucidating the cellular immune responses to mycobacterial proteins in leprosy and tuberculosis. Furthermore, the *M. tuberculosis* 65-kDa protein and four of the other mycobacterial proteins share homology with known eucaryotic and procaryotic stress proteins, some of which may play a role in autoimmunity.—Authors' Abstract

Mackworth-Young, C. G., Cairns, E., Sabbaga, J., Massicotte, H., Diamond, B., Bell, D. A. and Schwartz, R. S. Comparative study of idiotypes of monoclonal antibodies derived from patients with lupus and leprosy and from normal individuals. J. Autoimmun. **3** (1990) 415–429.

A collaborative study was performed to compare the expression of a series of idiotypes defined on human anti-DNA and other autoantibodies. Three panels of human monoclonal antibodies were tested: eight derived from patients with systemic lupus erythematosus (SLE); 13 from an individual with lepromatous leprosy; and 38 from normal subjects. The following rabbit anti-idiotype sera were used: one (RId16/6) raised against the lupus-derived monoclonal anti-DNA antibody 16/6, four (RId8E7, RId4G7, RId4D5 and RIdTH9) against leprosy-derived monoclonal antibodies of various specificities, and one (anti-4.6.3) against a normal-derived anti-DNA monoclonal (KIM 4.6). In addition, two other anti-idiotypes were used—one a murine monoclonal (31), the other a rabbit polyclonal (RId^D)—which had been raised against polyclonal anti-DNA antibodies from lupus serum. Further experiments were performed with immunoabsorbed fractions of RId8E7. Direct-binding and competition assays were used.

All of the anti-idiotypes produced different patterns of positivity among the three panels of human monoclonal antibodies, with the exception of RId8E7 and RId4G7, which showed considerable concordance. There was a tendency towards anti-idiotypes being disease- or group-specific: thus anti-4.6.3 failed to bind to any of the lupus or leprosy-derived monoclonals, while RId16/6 and RId8E7 bound most strongly to the lupus- and leprosy-derived antibodies, respectively. KIM 4.6 itself was bound only weakly by RId16/6, while 16/6 was not recognized by anti-4.6.3; 16/6 was, however, bound by 3I, while KIM 4.6 was not. 3I bound to several other monoclonals but RId^D, which has been shown to be specific for the anti-DNA fraction of lupus serum, did not bind to any of them. These results indicate that the majority of these anti-idiotype preparations recognize largely separate sets of determinants. The monoclonal antibodies which bind to DNA may be only partly representative of anti-DNA antibodies in the serum of lupus patients.—Authors' Abstract

Mahadevan, P. R. and Robinson, P. An antigenic complex that restores ability in leprosy patients to kill *Mycobacterium leprae*—the probable molecular events identified in *in vitro* experiments. Trop. Med. Parasitol. **41** (1990) 310–313.

The delipidified cell components of *Mycobacterium leprae* (DCC) obtained as an insoluble material was presented as an antigen by the macrophages of lepromatous leprosy patients. This resulted in *in vitro* lymphocyte proliferation and production of lymphokines, like IL-2 and IFN-γ. This DCC-induced culture supernatant was capable of activating patient macrophages, through changes induced in the membrane, as monitored by same specific markers, before and after exposure to the supernatant. The activated macrophages could recognize *M. leprae* as an antigen to initiate cell-mediated immunity and also recognize the bacilli as a pathogen to produce superoxide leading to the killing of phagocytosed *M. leprae*. Based on these observations, it is indicated that DCC could be a potent immunomodulatory restoring in the phagocytes of leprosy patients to kill *M. leprae* like normal resistant individuals.—Authors' Abstract

Mahon, A. C., Gebre, N. and Nurlign, A. The response of human B cells to *Mycobacterium leprae*. Identification of target antigens following polyclonal activation *in vitro*. Int. Immunol. **2** (1990) 803–812.

We have investigated the B-cell response to *Mycobacterium leprae* in leprosy patients and healthy controls. A comparison of Western-blotted proteins separated by two-dimensional gel electrophoresis and probed with pooled sera from LL and BT patients revealed distinct antigen recognition patterns for the two classifications of the disease. To characterize the circulating B cells capable of producing anti-*M. leprae* antibodies *in vitro*, peripheral blood lymphocyte cultures were activated polyclonally with an anti-CD3 monoclonal antibody. The resulting culture supernatants were used to probe Western-blotted *M. leprae* proteins and contained antibody reactive with a 10 kDa *M. leprae* antigen. This antibody was absent in stimulated culture supernatants from healthy occupational contacts or unexposed controls, suggesting the specificity of the response. Distinct repertoires of serum and culture supernatant anti-*M. leprae* antibodies were observed when Western-blotted antigens were probed after two-dimensional gel eletrophoresis. This method for assay of specific antibody production against individual components present in a complex mixture of antigens after polyclonal activation *in vitro* may be used to study the regulation of B-cell activation in leprosy and other diseases.—Authors' Abstract

Matsuo, K., Yamaguchi, R., Yamazaki, A., Tasaka, H., Terasaka, K., Totsuka, M., Kobayashi, K., Yukitake, H. and Yamada, T. Establishment of a foreign antigen secretion system in mycobacteria. Infect. Immun. **58** (1990) 4049–4054.

In order to develop recombinant *Mycobacterium bovis* BCG into a useful multivaccine vehicle, we established a foreign antigen secretion system in mycobacteria in which an extracellular α antigen of *M. kansasii* was utilized as a carrier. By using this system, a B-cell epitope (Glu-12-Leu-Asp-Arg-Trp-Glu-Lys-Ile-19) of human immunodeficiency virus type 1 pl^{gag}, which was identified by a fusion protein-based method, has been successfully obtained from BCG along with the α antigen. This is the first report of expression and secretion of a foreign viral antigen from BCG. It is possible that the system can become a universal vaccination vehicle applicable to protection against various infectious diseases.—Authors' Abstract

Munk, M. E., Gatrill, A. J. and Kaufmann, S. H. E. Target cell lysis and IL-2 secretion by γ/δ T lymphocytes after activation with bacteria. J. Immunol. **145** (1990) 2434–2439.

Peripheral blood T lymphocytes from healthy donors were stimulated with *Mycobacterium tuberculosis in vitro* and afterward analyzed phenotypically. Marked expansion of the γ/δ T cell population (3- to 21-fold) was observed in 15/21 donors 7 to 10 days after stimulation. In addition to *M. tuberculosis, M. leprae* (six of eight) as well

as the gram-positive bacteria, *Staphylococcus aureus* (two of six), group A streptococci (seven of nine), and *Listeria monocytogenes* (four of eight) augmented γ/δ TCR expression in peripheral blood T cells of many donors. γ/δ T lymphocytes expressed IL-2R and secreted IL-2 upon restimulation with *M. tuberculosis*. Stimulation with *M. tuberculosis* evoked specific cytolytic activities in γ/δ T lymphocytes because: γ/δ T cells lysed *M. tuberculosis* pulsed but not unpulsed targets; high concentrations of TCRδ1 monoclonal antibody facilitated killing of unpulsed target cells; and low doses of anti-TCRδ1 monoclonal antibody blocked killing of pulsed targets. Furthermore, γ/δ T cells from four donors, after activation with *M. tuberculosis* or with group A streptococci, respectively, only lysed targets pulsed with the homologous agents; whereas in other donors some crossreactivity was observed. We conclude that, upon contact with mycobacteria and perhaps other microorganisms, γ/δ T cells are activated which contribute to immunity against infection via IL-2 secretion and specific target cell lysis.—Authors' Abstract

Munno, I., Pellegrino, M., Fumo, G., Barbieri, G. and Jirillo, E. Studies on lymphokine production in lepromatous leprosy patients. Cytobios **62** (1990) 141–147.

In order to evaluate whether lymphokine (LK) release is impaired in patients with lepromatous leprosy (LL), the production of two LKs (induced by mitogens), namely, leukocyte inhibitory factor (LIF) and interleukin-2 (IL-2) from peripheral blood mononuclear cells of LL individuals was investigated. Results show that in eight patients CD4+ cells exhibit a reduced release of LIF, while CD8+ lymphocytes are still able to secrete this LK. In the remaining three patients both CD4+ and CD8+ cells produce LIF as do normal lymphocyte subpopulations. As far as IL-2 release is concerned, all patients fail to produce the above LK either using purified CD4+ or CD8+ lymphocytes. These data emphasize additional defects in immune responsiveness in leprosy.—Authors' Abstract

Narayanan, R. B., Girdhar, A. and Girdhar, B. K. CD1-positive epidermal Langerhans cells in regressed tuberculoid and lepromatous leprosy lesions. Int. Arch. Allergy Appl. Immunol. **92** (1990) 94–96.

A comparison was made of the numbers of epidermal Langerhans cells in active and regressed lesions of tuberculoid and lepromatous leprosy using the OKT6 and OKIa monoclonal antibodies. A reduction in the numbers of CD1+ epidermal Langerhans cells was noticed in the regressed lesions of both the tuberculoid and lepromatous leprosy lesions unlike the active lesions. The majority of infiltrates in both types of regressed lesions were HLA-DR+ and CD1−.—Authors' Abstract

Narayanan, R. B., Girdhar, A., Girdhar, B. K. and Malaviya, G. N. Immunohistological analysis of nerve granulomas in neuritic leprosy. Int. Arch. Allergy Appl. Immunol. **92** (1990) 50–55.

Immunohistological analysis of infiltrates of nerves in patients with neuritic leprosy was carried out using monoclonal antibodies defining T-cell subsets, Langerhans cells, HLA-DR antigens, and indirect immunofluorescence. In all, 8 nerves were analyzed; 2 of the 8 nerves showed epithelioid cell granulomas surrounded by large numbers of lymphocytes. The predominant lymphocytes in these granulomas were activated T cells expressing CD3 and HLA-DR antigens. The proportion of CD3+ and CD4+ cells was higher than that of CD8+ cells. The ratio of CD4+/CD8+ cells in these two biopsy specimens was 5.6 and 1.5, respectively. In these nerves CD4+ cells were diffusely scattered into epithelioid cell granulomas, while CD8+ cells were localized at the periphery of the granuloma. The remaining six nerves showed macrophages containing numerous bacilli, and a few lymphocyte and plasma cells diffusely distributed into the granuloma. In these nerves, only occasional lymphocytes expressing CD3 or CD4 or CD8 and HLA-DR antigens were noticed. In two of the biopsy specimens, a small proportion of CD8+ cells were visualized. Macrophages and Schwann cells were HLA-DR+ in all nerves. CD1+ cells were not seen in the infiltrates of any of these nerves. A similar pattern and distribution of cells was noticed in the nerve

granulomas of tuberculoid and lepromatous leprosy. These findings suggest that the mechanisms of nerve damage in the patients with neuritic leprosy could be either immunological or non-immunological, depending on the nature and characteristics of the infiltrates.—Authors' Abstract

Nathan, C., Squires, K., Griffo, W., Levis, W., Varghese, M., Job, C. K., Nusrat, A. R., Sherwin, S., Rappoport, S., Sanchez, E., Burkhardt, R. A. and Kaplan, G. Widespread intradermal accumulation of mononuclear leukocytes in lepromatous leprosy patients treated systemically with recombinant interferon γ. J. Exper. Med. **172** (1990) 1509–1512.

Intradermal administration of recombinant interferon γ (rIFN-γ) to lepromatous leprosy patients has converted the local histology toward a tuberculoid pattern. However, such changes have been confined to the site of injection. In contrast, in the present study, marked, intradermal accumulation of CD3+, CD4+, CD8+, and CD1a+ T cells and Leu-M5+ mononuclear phagocytes was induced at a distance from the sites of administration, in a dose-dependent manner, by 10 daily intramuscular injections of 10–30 μg rIFN-γ/m². Mononuclear cell infiltration began within 3 days of onset of rIFN-γ therapy and persisted at least 8 weeks. Intramuscular administration of rIFN-γ to lepromatous patients receiving concurrent chemotherapy can safely induce widespread histologic features of an upgrading reaction.—Authors' Summary

Ottenhoff, T. H. M., Walford, C., Nishimura, Y., Reddy, N. B. B. and Sasazuki, T. HLA-DQ molecules and the control of *Mycobacterium leprae*-specific T cell nonresponsiveness in lepromatous leprosy patients. Eur. J. Immunol. **20** (1990) 2347–2350.

The major histocompatibility complex (MHC) controls the outcome of the immune response to T-cell-dependent antigens by dictating whether T-cell responsiveness will result (MHC-immune response [Ir] genes) or alternatively T-cell nonresponsiveness will occur, possibly through the activation of suppressor cells (MHC-immune suppression [Is] genes). In mice, I-A molecules typically restrict antigen-specific helper-T cells. In contrast, H-2I-E molecules have been reported to control nonresponsiveness to a variety of antigens through antigen-specific suppressor cells. In analogy, HLA-DR molecules are the dominant restriction elements for helper-T cells in man. This forces the question whether DQ molecules may be involved in controling nonresponsiveness in man, e.g., through suppression. In one system, T-cell nonresponsiveness to *Schistosoma japonicum,* evidence has been presented supporting this notion. We have now used a second system, *Mycobacterium leprae*-specific T-cell nonresponsiveness, that is typically found in lepromatous (BL-LL) leprosy patients. We find positive but limited evidence for a role for HLA-DQ molecules in controling T-cell nonresponsiveness to *M. leprae* of the 22 nonresponder patients tested, 4 showed a proliferative T-cell response to *M. leprae* after the addition of DQ- but not DR-specific monoclonal antibody to the cell cultures. In one of the four BCG nonresponders, anti-DQ monoclonal antibody had a similar effect.—Authors' Abstract

Patil, S. A., Girdhar, B. K., Singh, K. P. and Sengupta, U. Detection of *Mycobacterium leprae* antigens in the sera of leprosy patients by sandwich immunoradiometric assay using monoclonal antibodies. J. Clin. Microbiol. **28** (1990) 2792–2796.

An immunological technique for demonstration of *Mycobacterium leprae* antigens in sera was developed by using specific as well as crossreactive monoclonal antibodies. The sandwich immunoradiometric assay which we developed is a simple, robust assay that is sensitive to the nanogram level. Sera from 72 leprosy patients were screened for the presence of antigen by this assay. A total of 69% of untreated tuberculoid leprosy patients showed 35-kDa antigen positivity, and 45% of these patients showed anti-35-kDa antibody positivity. Consistently higher antigen-positivity rates for the 35-, 12-, and 30- to 40-kDa components of *M. leprae* were observed in lepromatous leprosy patients than in tuberculoid leprosy patients. During the course of therapy the antigen-positivity rate gradually declined, and the antigen could not be detected in any of the 15 patients with sub-

sided cases of leprosy. Since antigen is presumably in excess before the antibody response is evoked, our experimental approach for antigen detection is likely to be useful by itself or along with antibody detection for diagnosis of early leprosy.—Authors' Abstract

Praputpittaya, K., Suriyanon, V., Hirunpetcharat, C., Rungruengthanakit, K. and Suphawilai, C. Comparison of IgM, IgG and IgA responses to *M. leprae* specific antigens in leprosy. Asian Pac. J. Allergy Immunol. **8** (1990) 19–25.

Antibodies of IgM, IgG, and IgA classes against *Mycobacterium leprae*-specific antigens (PGL-I, ND-O-BSA, and NT-O-BSA) were determined in the sera of 80 leprosy patients (28 untreated, 34 treated lepromatous, and 18 tuberculoid), 25 tuberculosis patients and 33 normal individuals of northern Thailand. No strong distinction in reactivity could be found between the three antigens. The IgM antibody assay yielded more positive results than assays for IgG and IgA. It was found that the positivity rates of IgM antibodies to all three antigens were highest in untreated lepromatous leprosy (82%). In tuberculoid leprosy, the positivity rates of IgM, IgG, and IgA to the antigens were more variable, ranging from 22% to 50%. Patients with tuberculosis and normal individuals did not produce IgM antibodies against the antigens. The results suggested that the determination of IgM against the three antigens is a more sensitive and specific test for active leprosy than those of IgG and IgA. The relationship between the duration of treatment and IgM antibody levels in lepromatous leprosy (LL) was studied. Untreated LL patients had significantly higher IgM and IgA antibody levels than treated patients. There was no difference in IgG antibody levels between the two groups, and the levels of both groups were higher than normal controls. Serial determination of IgM antibodies in 7 LL patients revealed that treatment was strongly associated with progressive decrease in IgM antibody levels against all three antigens.—Authors' Summary

Ramanathan, V. D., Parkash, O., Tyagi, P., Sengupta, U. and Ramu, G. Activation of the human complement system by phenolic glycolipid of *Mycobacterium leprae*. Microb. Pathogen. **8** (1990) 403–410.

The activation of the complement system by phenolic glycolipid-I (PGL) from *Mycobacterium leprae* was studied. It was found that PGL consumed hemolytic complement through both the classical and the alternative pathways. This was further studied at the level of C3. Although the activation was independent of anti-PGL antibodies present in normal human serum, the addition of antibody augmented the activation of complement by PGL. The uptake of C3 through the classical pathway was enhanced predominantly by IgM antibody; whereas IgG antibody against PGL was responsible for the augmentation of the alternative pathway activation. Furthermore, it was found that both the disaccharide and trisaccharide components of PGL were able to activate the complement system.—Authors' Abstract

Saad, M. H. F., Medeiros, M. A., Gallo, M. E. N., Gontijo, P. P. and Fonseca, L. S. IgM immunoglobulins reacting with the phenolic glycolipid-1 antigen from *Mycobacterium leprae* in sera of leprosy patients and their contacts. Mem. Inst. Oswaldo Cruz **85** (1990) 191–194.

For the first time in Brazil it was investigated the occurrence of IgM anti-PGL-I in the sera of household contacts of leprosy patients using the ELISA methodology. The sera of the multibacillary patients showed significantly more immunoreactivity than from the paucibacillary patients. It was observed a high subclinical infection incidence among household contacts (19.4%). The percentage of leprosy development was 5% (1/21) among the seropositive contact group. This finding suggests that serology could be useful as prognostic test, but for better definition it is necessary to test a population from an endemic area for long period of time.—Authors' Abstract

Sagaró Delgado, B., de la Rocha, A. D., Morales, M. C., Coffazo, S., Alonso, T. P., Diaz, M. A., Ochoa, C., Guillama, E., Bello, I. and López-Saura, P. [Systemic use

of interferon-gamma in lepromatous leprosy.] Rev. Leprol. Fontilles **17** (1990) 469–477. (in Spanish)

The existence of a specific defect in the immune response by the cells has already been observed in lepromatous leprosy. Therefore, we suggest the possibility of gamma-interferon so that it could influence the domain of the lepromatous immunological spectrum. A study has been performed to demonstrate if gamma-interferon could be an adjuvant of specific therapy in enabling the transformation or modification of the lepromatous leprosy immunological defect. Twenty patients who had never been under treatment or with not less than a 2-year period under specific therapy will be blindly randomized into two groups: one with gamma-interferon and the other with a placebo. Before and after the treatment the following studies are performed: histopathology, immunology and bacteriology. A preliminary communication is presented after opening the code for the first two patients included: one treated with gamma-interferon and another who received placebo. The response was much better in the first case from the bacteriological, histopathological and immunological points of view, even with negativization of bacilli detection.—Authors' English Summary

Saroyants, L. V., Yushchenko, A. A. and Alekseev, L. P. [Immunological characteristics in a different course of lepromatous leprosy.] Zh. Mikrobiol. Epidemiol. Immunobiol. **8** (1990) 70–72. (in Russian)

The study of the proliferative and regulatory functions of lymphocytes in patients with leprosy of the lepromatous type has shown that at the active stage of the disease both the response of lymphocytes to mitogens and their suppressor functions are decreased. During the regression of the disease these characteristics are restored to the normal level only in patients with the relapse-free course of the disease, while patients with relapses in their medical history retain the low level of such characteristics. It is expedient to use these cell-mediated immunity characteristics as signs permitting the formation of risk groups of patients who may expect the relapse of the disease.—Authors' English Abstract

Sulçebe, G. and Nakuçi, M. Anti-phenolic glycolipid 1 IgM antibodies in leprosy patients and in their household contacts. Lepr. Rev. **61** (1990) 341–346.

Although they have no apparent protective action, the specific antibodies are important markers of the infection with *Mycobacterium leprae.* For their detection, we employed an ELISA method using as substrate a synthetic immunodominant disaccharide of phenolic glycolipid I antigen of *M. leprae,* conjugated with bovine serum albumin (D-BSA). Increased levels of anti-D-BSA antibodies of the IgM class were detected in 61.5% of the 13 leprosy patients and in 13.3% of their 53 household contacts; whereas they were not found in any of the 37 normal blood donors. A strong correlation ($r = -0.846$) was found between the antibody levels and the duration of the disease among the 12 patients with lepromatous leprosy. These preliminary data demonstrate the usefulness of this method for epidemiological studies and for the detection of cases with subclinical infection.—Authors' Summary

Theetranont, C., Bhoopat, L. and Scollard, D. M. Cell changes and soluble interleukin-2 receptors (Tac peptide) in leprosy reversal reactions using suction-induced blisters. J. Med. Assoc. Thai. **73** (1990) 181–190.

The cellular contents and soluble interleukin-2 receptor (IL-2R) [Tac peptide] of skin blisters induced by suction over 7 reversal reaction (RR) patients were examined using immunoperoxidase and ELISA techniques, respectively. The helper-T activity (CD4+ cells) and helper : suppressor ratio were significantly greater in borderline lepromatous (BL) lesions with RR than in quiescent BL lesions. Interestingly, the intracutaneous levels of Tac peptide were elevated and directly correlated with the increases in CD4+ cells. The systemic administration of corticosteroids revealed a reduction in the numbers of CD4+ cells in the lesions. These results indicate that RRs are manifestations of a spontaneous in-

crease in delayed-type hypersensitivity (DTH) and possibly cell-mediated immunity (CMI) in leprosy patients. The mechanism of such changes in immunity is of considerable value in understanding reversal reactions and the underlying determinants of DTH and CMI in leprosy and this, in turn, will have a bearing on the potential for proposed vaccines or immunotherapy.—Authors' Summary

Thomas, B. M. and Mukherjee, R. Antineural antibodies in sera of leprosy patients. Clin. Immunol. Immunopathol. **57** (1990) 420–429.

A microtiter plate ELISA with semipurified human nerve sonicate antigen(s) (NA) was used to screen the sera of leprosy patients. High titers of IgG and low titers of IgM classes of antineural antibodies directed to peripheral nerve antigens were detected in LL, BL, BB, BT, and TT categories of leprosy. In the Western blot, leprosy sera recognized 50- to 55-, 85- and 108-kDa molecular weight protein bands of NA. The identity of these protein bands immunoreactive with leprosy sera was checked with a panel of commercially available antibodies to known neural proteins. The 50- to 55-kDa band reacted with anti-S100 and antiglial fibrillary acidic protein antibodies, while 85 and 108 kDa could not be identified. Whole immunoglobulins isolated from leprosy sera with high titers of antineural antibodies induced cytotoxicity of the cultured glial cell line in the presence of complement.—Authors' Abstract

Waters, M. F. R and Ridley, D. S. Tuberculoid relapse in lepromatous leprosy. Lepr. Rev. **61** (1990) 353–365.

It is commonly accepted that the attainment of bacteriological negativity fails to restore the immune state of leprosy patients who have downgraded to lepromatous. We report six patients who had been lepromatous (LLs), and who, after many years of chemotherapy and bacteriological negativity, were found upon relapse to have upgraded to borderline-tuberculoid (BT). Five had become Mitsuda-lepromin positive. The relapses could be accounted for by proven or suspected dapsone resistance. The upgrading was associated with minimal signs of reaction, which was attributed to the low level of antigen in the almost resolved lesions. The manner of development of the new high immune lesions resembled the onset of a primary infection, clinically and histologically. The development of a positive Mitsuda reaction in longstanding LL leprosy is not necessarily an indication of cure.—Authors' Summary

Waters, M. F. R., Ridley, D. S. and Lucas, S. B. Positive Mitsuda lepromin reactions in long-trem treated lepromatous leprosy. Lepr. Rev. **61** (1990) 347–352.

Twenty-four lepromatous (LL) patients, treated for 22 to 40 years with chemotherapy, including sulfones and with multidrug therapy, were tested with standard Wade-Mitsuda lepromin. Thirteen gave weak positive (3–4 mm) Mitsuda reactions, confirmed histologically in the 10 whose reactions were biopsied. Six of the 11 negative reactors were partly accounted for by a history of relapse, and 2 others had probably taken dapsone irregularly. Eleven control LL patients, treated for less than 20 years, were uniformly lepromin negative. Spontaneous lepromin conversion appears to occur around 24 years after commencing successful chemotherapy. The late Mitsuda conversions are attributed to delayed clearance of the reservoir of bacterial antigen, but a poor correlation between Mitsuda and Fernandez positivity is not explained.—Authors' Summary

Wiley, E. L., Mulhollan, T. J., Beck, B., Tyndall, J. A. and Freeman, R. G. Polyclonal antibodies raised against bacillus Calmette-Guerin, *Mycobacterium duvalii,* and *Mycobacterium paratuberculosis* used to detect mycobacteria in tissue with the use of immunohistochemical techniques. Am. J. Clin. Pathol. **94** (1990) 307–312.

Commercially available polyclonal antibodies raised against strains of mycobacteria were used to detect organisms in tissue sections from 34 cases of tuberculosis, leprosy, and atypical mycobacteria. Thirty-two cases of fungal infections, granulomatous inflammation, and sarcoidosis were used as

negative controls. Sections stained with the use of antibodies raised against bacillus Calmette-Guerin (BCG), *Mycobacterium duvalii* (MD), and *M. paratuberculosis* (MP) were compared with Kinyoun and Fite-stained tissue sections. In caseating granulomata, clumps of mycobacterial debris, cells, and cell fragments stained. In histiocytic granulomata of mycobacterial infections, histiocyte cytoplasm contained both organisms and debris. The three antibodies showed crossreactivity against the four groups of mycobacteria tested. Mycobacterial staining using immunoperoxidase was apparent in most cases at low-power (scanning) magnification. Thirty-two of 34 cases of mycobacterial infection, including all 24 Kinyoun-Fite-positive cases, were positive for immunoreactive organisms and debris using anti-MD, anti-BCG, and/or anti-MP. Eight of 10 cases of culture-proven mycobacterial infection, in which Kinyoun and Fite stains were negative, had immunoreactive organisms or antigen with anti-BCG, MD, or MP. The antibodies also stained organisms in five cases of sporotrichosis in which the organisms were identified as yeast forms in tissue sections.—Authors' Abstract

Microbiology

Bhatia, V. N., Vanaja, G., Rao, S. and Elango, T. V. Some observations on skin smear examination. Indian J. Lepr. **62** (1990) 338–345.

Forty slit and scrape smears in a planned study and 35 routine smears picked up from the laboratory were examined. An end-to-end examination of the smears detected additional positives and gave a higher bacterial index (BI) than what was reported in the routine. Acid-fast bacilli were found to be distributed in only 1% to 3% of the fields in the smears. The bacilli were found mostly in the center and in narrow bands between the center and periphery of the smears. Some of the high BI smears were found to contain areas completely free from bacilli.—Authors' Abstract

Garbe, T., Jones, C., Charles, I., Dougan, G. and Young, D. Cloning and characterization of the *aroA* gene from *Mycobacterium tuberculosis*. J. Bacteriol. **172** (1990) 6774–6782.

The *aroA* gene from *Mycobacterium tuberculosis* has been cloned by complementation of an *aroA* mutant of *Escherichia coli* after lysogenization with a recombinant DNA library in the λgt11 vector. Detailed characterization of the *M. tuberculosis aroA* gene by nucleotide sequencing and by immunochemical analysis of the expressed product indicates that it encodes a 5-enolpyruvylshikimate-3-phosphate synthase that is structurally related to analogous enzymes from other bacterial, fungal, and plant sources. The potential use of the cloned gene in construction of genetically defined mutant strains of *M. tuberculosis* by gene replacement is proposed as a novel approach to the rational attenuation of mycobacterial pathogens and the possible development of new antimycobacterial vaccines.—Authors' Abstract

Hermans, J., Boschloo, J. G. and de Bont, J. A. M. Transformation of *Mycobacterium aurum* by electroporation: the use of glycine, lysozyme and isonicotinic acid hydrazide in enhancing transformation efficiency. FEMS Microbiol. Lett. **72** (1990) 221–224.

A transformation procedure for *Mycobacterium aurum* using electroporation was developed and optimized. Effects of glycine and lysozyme treatments were studied, and isonicotinic acid hydrazide was shown to increase transformation efficiency tenfold.—Authors' Summary

Ishaque, M. Growth of *Mycobacterium leprae* under low oxygen tension. Microbios **64** (1990) 7–17.

Despite numerous attempts, *Mycobacterium leprae* has yet to be cultivated *in vitro*. This organism has been considered as mi-

croaerophilic. The effects of various known gas mixtures on the *in vitro* growth of *M. leprae* were investigated. A gas mixture containing 2.5% O_2 and 10% CO_2 was found to be more favorable for the growth of this mycobacterium on artificial medium. Growth was evaluated by three parameters, namely, cell counts, bacterial ATP and DNA. An optimal growth of *M. leprae,* as determined by all three parameters, on both liquid and solid media was obtained between 18 and 24 weeks of incubation under optimal gas mixture. Solid medium which contained egg yolk was relatively more beneficial for *in vitro* growth than the liquid medium. The cultivated bacilli exhibited some important characteristics specific for *M. leprae,* including growth in mouse foot pads. The bacilli gradually lost their power of adaptation to grow on artificial media and did not show any ATP or DNA after about 36 weeks of incubation.—Author's Abstract

Pal, D., Chakrabarty, A. N. and Dastidar, S. G. Is leprosy bacillus a chemo-autotrophic nocardio-form organism? Indian J. Lepr. **62** (1990) 351–357.

Numerous attempts at *in vitro* cultivation of the leprosy bacillus have all proved to be unsuccessful. Recently, we have repeatedly isolated chemo-autotrophic nocardio-form (CAN) organisms in pure culture from multibacillary cases of leprosy. We find that these resemble the leprosy bacillus in many respects and suggest that the leprosy bacillus may be closer to the genus *Nocardia* than to *Mycobacterium,* and that it may be a chemo-autotroph, requiring only simple sources of carbon and nitrogen for its growth. This is in contrast to most other human pathogens, which are heterotrophs requiring complex sources of carbon and nitrogen for their growth. This could offer a possible explanation for the repeated failure at *in vitro* cultivation of the leprosy bacillus.—Authors' Abstract

Puzo, G. The carbohydrate- and lipid-containing cell wall of mycobacteria, phenolic glycolipids: structure and immunological properties. Curr. Rev. Microbiol. **17** (1990) 305–327.

Phenolic glycolipids were first discovered as cell-wall constituents of *Mycobacterium bovis, M. bovis* BCG, *M. marinum,* and *M. kansasii.* Recently, such compounds were also isolated from *M. leprae* and have been shown to be specific-species serological markers. Moreover, they seem to be involved, in the case of lepromatous leprosy, in the stimulation of the suppressor-T cells. The functional activities of these phenolic glycolipids over the immune cells stimulation emphasized the role played by these molecules in the mycobacteria pathogenicity. Phenolic glycolipids have also been found in *M. gastri* and *M. tuberculosis* strain *Canetti.* From a structural point of view, these glycolipids contain the same aglycon moiety mainly assigned to phenolphthiocerol diester while the sugar part structure confers to some of these glycolipids their antigenic specificity. The search of immunoreactive glycolipids and their function analysis remain a challenge for chemists and immunologists for the understanding of the mycobacteria pathogenicity.—Authors' Abstract

Rastogi, N. and Hellio, R. Evidence that the capsule around mycobacteria grown in axenic media contains mycobacterial antigens: implications at the level of cell envelope architecture. FEMS Microbiol. Lett. **70** (1990) 161–166.

The intracellular growth of pathogenic mycobacteria has been linked to the presence of an electron transparent zone (ETZ or capsule), which surrounds the phagocytized bacteria and prevents the diffusion of lysosomal enzymes in infected macrophages. Recently, it was suggested that this capsule may be a bacterial structure, even being present in test-tube-grown pathogenic mycobacteria. In the present paper, we show that under special fixation and embedding conditions, this capsule was clearly observed among 7 strains of mycobacteria grown in axenic media and also in *Mycobacterium leprae* extracted and purified from experimentally infected armadillo or nude mice. In the case of bacteria treated likewise but subject to a prior dehydration step, this capsular structure disappeared, suggesting its lipidic nature. Ultrathin sections of *M. intracellulare* after immunolabeling showed

for the first time that this capsule obtained mycobacterial antigens confirming its mycobacterial origin. It is suggested that the mycobacterial capsule may be formed of inert lipids, in which surface antigens are embedded.—Authors' Summary

Sampson, J. S., O'Connor, S. P., Holloway, B. P., Plikaytis, B. B., Carlone, G. M. and Mayer, L. W. Nucleotide sequence of *htpB,* the *Legionella pneumophila* gene encoding the 58-kilodalton (kDa) common antigen, formerly designated the 60-kDa common antigen. Infect. Immun. **58** (1990) 3154–3157.

Gene *htpB,* which encodes the 58-kilodalton protein of *Legionella pneumophila,* was cloned in *Escherichia coli* and its complete nucleotide sequence was determined. Analysis of this sequence revealed an open reading frame of 1644 nucleotides encoding a protein with a predicted molecular mass of 57,952 daltons. Data obtained by amino-terminal sequencing of the purified 58-kDa protein agreed, except for one amino acid residue, with the predicted amino acid sequence, identifying this open reading frame as *htpB.* A comparison of the primary structure of this protein to other proteins of similar molecular weights from *E. coli, Mycobacterium leprae, M. tuberculosis,* and *Coxiella burnetii* revealed significant regions of sequence similarity, which are discussed.—Authors' Abstract

Snapper, S. B., Melton, R. E., Mustafa, S., Kieser, T. and Jacobs, W. R., Jr. Isolation and characterization of efficient plasmid transformation mutants of *Mycobacterium smegmatis.* Mol. Microbiol. **4** (1990) 1911–1919.

Recent development of vectors and methodologies to introduce recombinant DNA into members of the genus *Mycobacterium* has provided new approaches for investigating these important bacteria. While most pathogenic mycobacteria are slow-growing, *Mycobacterium smegmatis* is a fast-growing, nonpathogenic species that has been used for many years as a host for mycobacteriophage propagation and, recently, as a host for the introduction of recombinant DNA. Its use as a cloning host for the analysis of mycobacterial genes has been limited by its inability to be efficiently transformed with plasmid vectors. This work describes the isolation and characterization of mutants of *M. smegmatis* that can be transformed, using electroporation, at efficiencies 10^4 to 10^5 times greater than those of the parent strain, yielding more than 10^5 transformants per μg of plasmid DNA. The mutations conferring this efficient plasmid transformation (Ept) phenotype do not affect phage transfection or the integration of DNA into the *M. smegmatis* chromosome, but seem to be specific for plasmid transformation. Such Ept mutants have been used to characterize plasmid DNA sequences essential for replication of the *M. fortuitum* plasmid pAL5000 in mycobacteria by permitting the transformation of a library of hybrid plasmid constructs. Efficient plasmid transformation of *M. smegmatis* will facilitate the analysis of mycobacterial gene function, expression and replication and thus aid in the development of BCG as a multivalent recombinant vaccine vector and in the genetic analysis of the virulence determinants of pathogenic mycobacteria.—Authors' Summary

Sritharan, M. and Ratledge, C. Iron-regulated envelope proteins of mycobacteria grown *in vitro* and their occurrence in *Mycobacterium avium* and *Mycobacterium leprae* grown *in vivo.* Biol. Metals **2** (1990) 203–208.

Several iron-regulated envelope proteins (IREPs), 11–180 kDa, have been detected in preparations of walls and membranes of *Mycobacterium smegmatis,* in an armadillo-derived mycobacterium (ADM) and in *M. avium.* The same sized proteins from *M. vaccae* appeared under both iron-deficient and iron-sufficient growth conditions. Two larger proteins, of 240 and 250 kDa, appeared in the membranes of *M. smegmatis* and *M. avium* only when grown iron-sufficiently but were constitutively present in both ADM and *M. vaccae.* The IREPs from *M. smegmatis* were not induced under zinc-deficient growth conditions. Three of the four IREPs (14, 21 and 29 kDa) recognized in *M. avium* grown *in vitro* were also recovered from membrane fractions of the same strain grown in mice. In addition, these

membranes contained both the high-molecular-mass proteins associated with iron-sufficient growth conditions. Membranes of *M. leprae,* recovered from infected armadillos, showed the faint presence of a possible IREP at 29 kDa, and wall preparations showed the presence of a 21-kDa protein. Membranes also contained the two larger proteins at 240 and 250 kDa. An explanation for the simultaneous occurrence of both low-iron-regulated and high-iron-regulated proteins is offered.—Authors' Summary

Sut, A., Sirugue, S., Sixou, S., Lakhdar-Ghazal, F., Tocanne, J.-F. and Lanéelle, G. Mycobacteria glycolipids as potential pathogenicity effectors: alteration of model and natural membranes. Biochemistry **29** (1990) 8498–8502.

Four mycobacterial wall glycolipids were tested for their effects on phospholipidic liposome organization and passive permeability and an oxidative phosphorylation of isolated mitochondria. From fluorescence polarization of diphenylhexatriene performed on liposomes it was concluded that the two trehalose derivatives (dimycoloyltrehalose and polyphthienoyltrehalose) rigidified the fluid state of liposomes, the triglycosyl phenolphthiocerol slightly fluidized the gel state, while the peptidoglycolipid ("apolar" mycoside C) just shifted the phase transition temperature upward. Dimycoloyltrehalose was without effect on liposome passive permeability, as estimated from dicarboxyfluorescein leak rate, and polyphthienoyltrehalose and triglycosyl phenolphthiocerol slightly decreased leaks, while mycoside C dramatically increased leaks. Activity of these lipids on mitochondrial oxidative phosphorylation was examined. The two trehalose derivatives have been tested previously: both had the same type of inhibitory activity, dimycoloyltrehalose being the most active. Triglycosyl phenolphthiocerol was inactive. Mycoside C was very active, with effects resembling those of classical uncouplers: this suggested that its activity on mitochondria was related to its effect on permeability. All these membrane alterations were called nonspecific because it is likely that they result from nonspecific lipid–lipid interactions, and not from recognition between specific molecular structures. Such nonspecific interactions could be at the origin of some of the effects of mycobacteria glycolipids on cells of the immune system observed in the last few years.—Authors' Abstract

Williams, D. L., Gillis, T. P. and Portaels, F. Geographically distinct isolates of *Mycobacterium leprae* exhibit no genotypic diversity by restriction fragment-length polymorphism analysis. Mol. Microbiol. **4** (1990) 1653–1659.

Differentiation of microorganisms for taxonomic purposes is based primarily on phenotypic characteristics, which are the direct or cumulative result of gene expression. Since expression of phenotypic characteristics usually relies on *in vitro* growth of a microorganism, noncultivable organisms, such as *Mycobacterium leprae,* present major problems for the identification of potential variants based on phenotypic similarities or differences between individual isolates. We have employed the use of restriction fragment-length polymorphism (RFLP) analysis of chromosomal DNA of *M. leprae* isolates, including human isolates from geographically distinct regions of the world and isolates from a sooty mangabey monkey and an armadillo, to assess the relatedness among these isolates. Restriction endonuclease (*Eco*RI, *Bst*EII, *Pst*I, and *Pvu*I) digests of chromosomal DNA were analyzed using DNA probes encoding all or part of the 12-kDa, 18-kDa, 28-kDa, 65-kDa and 70-kDa proteins of *M. leprae* as well as a probe containing an *M. leprae*-specific sequence repeated up to 20 times in the *M. leprae* chromosome. Comparison of the resulting autoradiographs showed that the RFLP patterns were all identical, indicating that these isolates contained no polymorphism with respect to the restriction endonuclease sites analyzed. In addition, RFLP patterns of two separate human *M. leprae* isolates remained unchanged after three cycles of experimental infection in the armadillo model. These results indicated that the *M. leprae* isolates tested in this study were indistinguishable at the genotypic level, strongly suggesting homogeneity among members of this species.—Authors' Summary

Woods, S. A. and Cole, S. T. A family of dispersed repeats in *Mycobacterium leprae.* Mol. Microbiol. **4** (1990) 1745–1751.

The genome of the causative agent of leprosy, *Mycobacterium leprae,* contains at least 28 copies of a dispersed repetitive sequence, RLEP. From nucleotide sequence analysis it was clear that the RLEP element consists of a 545bp central domain flanked by a 100bp left-end and a 44bp right-end, sometimes associated with a 47bp extension. The presence of the left and right ends is variable, and this allowed three different RLEP configurations to be defined. When the polymerase chain reaction was used to study variation of the central region at least 12 different classes were detected, suggesting that no two RLEP sequences may be identical. Furthermore, they have few features in common with classical bacterial insertion sequences.—Authors' Summary

Experimental Infections

Banerjee, R., Chaudhury, S. and Hati, A. K. Transmission of *Mycobacterium leprae* from lepromatous leprosy patients to the skin of mice through intermittent feeding. Trop. Geogr. Med. **42** (1990) 97–99.

Batches of hungry *Aedes aegypti* mosquitoes which partially sucked blood from the skin lesions of proved untreated lepromatous leprosy (LL) patients were allowed immediately to feed on a portion of the skin of a cleanly shaved Swiss mouse. The portion of the skin was cut, homogenized on the same day, and extracted with chloroform. Out of 10 extracts, stained for acid-fast bacilli (AFB), *Mycobacterium leprae* were demonstrated in eight, indicating transfer of bacilli mechanically to the biting spot through intermittent feeding. Out of 50 probosces dissected and stained for AFB, *M. leprae* were demonstrated in 45.—Authors' Abstract

Boddingius, J. and Dijkman, H. Subcellular localization of *Mycobacterium leprae*-specific phenolic glycolipid (PGL-I) antigen in human leprosy lesions and in *M. leprae* isolated from armadillo liver. J. Gen. Microbiol. **136** (1990) 2001–2012.

Phenolic glycolipid (PGL-I), an antigen specific to *Mycobacterium leprae,* was localized subcellularly in *M. leprae* residing in human skin, in *M. leprae* isolated from armadillo liver ("isolated *M. leprae*"), and outside *M. leprae* in human lepromatous skin. For a quantitative localization of PGL-I sites, specimens, including skin segments stored for 6 years in glutaraldehyde, were embedded in hydrophilic Lowicryl (K_4M) resin for ultrathin sectioning. Ultracryosections and Araldite sections of comparable specimens were used for comparison of localization results. A monoclonal antibody (F 47-21-3) directed to antigenic oligosaccharide of PGL-I was employed as primary antibody in immunogold labeling of ultrathin sections. K_4M-immunogold methods gave very satisfactory quantitive gold-labeling of PGL-I. The localization of PGL-I by this method partially corresponded with sites detectable in both ultracryosections and the qualitatively superior Araldite sections, but new sites were also localized. Cell walls in human *M. leprae* and in isolated *M. leprae* possessed many PGL-I sites, particularly in dividing organisms. PGL-I or its antigenic oligosaccharide was also found, to a lesser extent, in the bacterial cytoplasm. Capsules discernible around part of isolated *M. leprae* cells displayed heavy PGL-I labeling, sometimes clearly confined to a zone distant from the cell wall. Extrabacterial PGL-1 in *M. leprae*-infected human skin was encountered 1) in phagolysosomes and cytoplasm proper of dermal macrophages containing *M. leprae,* and 2) intra- and extracellularly in epidermal areas where basal cells harbored *M. leprae* in untreated multibacillary patients.—Authors' Abstract

Hussein, N., Ostler, B., Gormus, B. J., Wolf, R. and Walsh, G. P. Intraocular pressure changes and postural changes of intraocular pressure in experimentally induced Hansen's disease of rhesus, mangabey, and African green monkeys. Br. J. Ophthalmol. **74** (1990) 647–649.

In our long-term evaluation of patients with Hansen's disease we have frequently found reduction of their intraocular pressure. Furthermore, we noted changes in their intraocular pressure on change of posture. To determine if these changes have any significance we measured the intraocular pressures of 24 experimentally infected and 39 control monkeys in both sitting and reclining positions. We found significant reduction of intraocular pressure in 66.7% compared with controls in the sitting position, and a significant increase in intraocular pressure in 79% when checked first in the sitting then in the reclining position. We offer a possible pathophysiological explanation as to why the changes occur.—Authors' Abstract

Richard, L., Forget, A. and Turcotte, R. Biological properties of factors secreted by antigen-reactive suppressor cells in mice infected with *Mycobacterium lepraemurium.* Infect. Immun. **58** (1990) 3531–3536.

Antigen-reactive cells were isolated from the spleens of *Mycobacterium lepraemurium*-infected C57BL/6 mice on petri dishes coated with mycobacterial antigens. When adoptively transferred to syngeneic mice, the mycobacterial antigen-reactive cells were found to depress the induction and expression of the delayed-type hypersensitivity (DTH) reaction to *M. lepraemurium* antigens. The adoptive transfer of soluble suppressor factors (SF) secreted by these cells inhibited only the expression of DTH. The cells depressing the induction of DTH mainly belonged to the L3T4+ (CD4+) T-lymphocyte subset; whereas those depressing its expression differed from the L3T4+ and Lyt-2+ (CD8+) subsets. Treatment of *M. lepraemurium*-infected mice with SF reduced their mean survival time and enhanced the multiplication of bacilli at the site of infection and their dissemination to the spleen and liver. *In vitro* at least, SF appeared to interfere at the level of mycobacterial antigen recognition by T lymphocytes rather than at the levels of antigen processing and presentation by macrophages.—Authors' Abstract

Zhou, H.-M., *et al.* [Severity of leprosy eye lesions in armadillos infected with *M. leprae.*] China Lepr. J. **6** (1990) 127–131. (in Chinese)

The pathology in 127 eyes of 66 armadillos infected experimentally with *Mycobacterium leprae* in three research institutes was studied. Although all the animals were infected with almost the same amount of bacilli intravenously, the proportions of the numbers of animals with severe and mild lesion in the eyes in these three groups, from Forshungsinstitut Borstel, Florida, and from Amsterdam, were not similar.

For the Borstel and Florida groups, the percentage of animals with mild lesions were 82% and 87%, and those with severe lesions were 18% and 13%, respectively. No meaningful differences were found between the duration of infection and the amount of the bacilli in the livers and spleens and the severity of the lesions in the eyes of these animals.

In the animals from Amsterdam, the percentage of animals with severe eye lesions was 80%; those with mild lesions was 20%. Although no significant difference between the severity of the eye lesions, the duration of infection, and the bacillary content in the liver was found, the severity of the eye lesions in these animals was parallel to the amount of bacilli in their spleens.

Some differences in manipulation of the infection of the animals in these three institutes were found. The main differences were: all the animals of the Amsterdam group were inoculated on the same day and the bacilli were obtained from the spleen of an armadillo from Borstel. The suspension of bacilli had not been subjected to freezing and thawing before inoculation but had been subjected to ultrasonicating just prior to inoculation. More uniform severe lesions of the eyes occurring in this group might be related to these differences.—Authors' English Abstract

Epidemiology and Prevention

Al-Kandari, S., Al-Anezi, A., Pugh, R. N. H., Al-Qasaf, F. and Al-Abyad, S. Leprosy in Kuwait: an epidemiological study of new cases. Ann. Trop. Med. Parasitol. **84** (1990) 513–522.

The latency of infection in leprosy is long, so that new cases may present several years after emigration from endemic areas. This is of concern to the health authorities in Kuwait, since there is a sizable immigrant population. An epidemiological study of new cases was, therefore, conducted to assess the extent of the leprosy problem. A total of 121 (99 male, 22 female) consecutive new leprosy patients were diagnosed nationwide over a 6-year period (1983–1988). Over 95% of the patients were foreign born, emphasizing that the problem in Kuwait is mainly a reflection of immigration patterns. There were 74 cases of Asio-Indian origin, 13 Oriental, and 34 Arab (including 2 Kuwaiti). This represents a respective mean incidence of the disease in Kuwaitis and other nationalities of 0.49 and 18.92 per 100,000 per year. Polar lepromatous (LL) leprosy was the most frequent type in the Arab group (44.1%), and polar tuberculoid (TT) the most frequent in the Asio-Indian group (37.8%). LL and borderline lepromatous (BL) types of leprosy were significantly more frequent in patients over 45 years old and in females ($p < 0.05$), contributing to the higher rate of LL in the Arab cases. The mean lag time from symptoms onset to presentation to doctor was 9.4 (0–192) months, with lepromatous cases tending to present later than other types. The longest lag times occurred in Arab women with LL, suggesting that cultural influences may delay presentation of leprosy. The mean interval from presentation to diagnosis was 4.1 weeks. The mean latency from entry into Kuwait to diagnosis was 44.7 (range 0–180) months, which stresses the need for physicians to remain vigilant in considering leprosy, especially in any patient with dermatological, neurological or ophthalmic manifestations of disease.—Authors' Abstract

Edorh, A. A., Gerardin, M. and Kpadenou, Y. K. [The leprosy endemic in the Golfe Prefecture of Togo in 1987.] Afr. Med. **28** (1989) 542–546. (in French)

After the multidrug therapy of "accessible" patients and prior the multidrug therapy "for all," still with the WHO 1981 schemes, a better knowledge of certain parameters of the leprosy endemic in the Golf prefecture is required.

Likewise, the present situation is characterized by a prevalence of 0.41 per thousand; 43.2% of the patients are multibacillary; 8.5% children; 22.7% children are multibacillary; 57.1% of the patients in account are men, 42.9% women. Out of 259 registered patients, 152 are under treatment, 33 new patients were detected in 1987. Among them, 27% are of the L form; 9% of the B form; 61% of the T form and 3% of the I form, which means 36% multibacillaries against 64% paucibacillaries. The incidence is of 0.052 per thousand; 12.1% of children and 15% of crippled are numbered among the new patients.—Authors' English Summary

Ibrahim, M. A., Kordy, M. N., Aiderous, A. H. and Bahnassy, A. Leprosy in Saudi Arabia, 1986–89. Rev. **61** (1990) 379–385.

This study on leprosy includes information obtained from the Ibn Sina Hospital, a specialized center established 27 years ago for treatment and management of the disease in Saudi Arabia. A total of 792 patients with leprosy were reported during the period of the study (1986–1989). A steady decline was observed in the number of patients reported: 432 (54.55%) were non-Saudi and 360 (45.45%) were Saudi. Patients were reported from a total of 22 different countries. The majority of the non-Saudi patients were from the Yemen, 286 (36.11%). The male-to-female ratio was 3.83:1. The age groups comprised: 133 (16.79%), 51 to 80; 575 (72.60%), 21 to 50; and 84 (10.61%), under 20 years of age. The disease was classified into five categories (Ridley and Jopling classification): 295 (37.25%), lepromatous type; 238 (30.05%), tuberculoid type; 146 (18.43%), borderline-

tuberculoid type; 29 (3.66%), borderline type; and 84 (10.61%), borderline-lepromatous type.

Although the number of registered patients is decreasing, this trend does not suggest an overall decline in the disease in the country. It is recommended, therefore, that the services being provided to patients with leprosy must be integrated with the nationwide network of the primary health care centers to implement effective control and prevention, including health education for the general population. Furthermore, mutual agreements must be developed with adjacent countries to study the geographic distribution of the disease.—Authors' Summary

Rodríguez Idígoras, M. I. and Petit, C. [Leprosy in Andalucía.] Rev. Leprol. Fontilles **17** (1990) 577–587. (in Spanish)

Andalucía is the Autonomous Community of Spain that has the largest number of leprosy patients estimated in 3000 cases, although only 1582 are registrated. The prevalence in this community is of 0.26 cases per 1000 inhabitants. The distribution of the patients in the region is irregular because 75% of the cases belong to the Oriental part of the community, while in the Occidental part there are only 25%. The actual distribution of the patients according to provinces presents the following information: the largest number of patients belongs to Málaga and Jaén, these provinces presenting a prevalence rate of 0.61 cases per 1000 inhabitants. The clinical classification of the patients reveals a proportion of lepromatous to tuberculoid of 2.5 to 1. The distribution according to sex is slightly higher in men with 56.38% of the cases and 50% of the patients are between 50 and 70 years of age. The incidence in the last 10 years reflects that the number of cases detected in Andalucía represent 50% of the total of cases detected in Spain. At the present moment there exists the need to incorporate these patients into the primary health care system together with other specialized groups that will support the activities of the primary health care system.—Authors' English Summary

Saha, K., Rao, K. N., Chattopadhya, D., Lakshmi, V., Gadi, S. and Dutta Banik, N. D. A study on nutrition, growth and development of a high-risk group of children of urban leprosy patients. Eur. J. Clin. Nutr. **44** (1990) 471–479.

This paper describes a cross-sectional study of the physical development of a high-risk group of 182 socially deprived, healthy children of leprosy patients ranging from preschool age to early teens. They were rescued at the age of 4 years from the distress of leprosy colonies where they were born, and brought up in government homes (preventorium) under better environmental conditions. Of them, 135 children could be followed clinically for 10 years for the development of childhood leprosy. Another 94 children of leprosy patients living with their parents were included for comparison. A group of 158 normal children of similar economic status and age group were included as controls. It was observed that, although better environment, food and training were provided in the preventorium, so that the children could be brought into the national mainstream, nevertheless 5 children developed an indeterminate type of leprosy during the course of 10 years. This is the first report of growth and development of children of leprosy patients from the Indian subcontinent.—Authors' Abstract

Rehabilitation

Birke, J. A., Novick, A., Graham, S. L., Coleman, W. C. and Brasseaux, D. M. Methods of treating plantar ulcers. Phys. Ther. **71** (1991) 116–122.

The purpose of this article is to describe the indications, precautions, and fabrication techniques for orthotic devices the authors use to facilitate the healing of plantar

ulcers. The methods of fabricating and applying three types of orthotic devices developed by the staff at the Gillis W. Long Hansen's Disease Center, Carville, Louisiana, U.S.A.—walking casts, walking splints, and cutout sandals—are described. Patient examples are given for each of the methods. These techniques, in conjunction with patient education and the use of special footwear, provide clinicians with procedures they can use to aid in the healing of plantar ulcers secondary to leprosy, diabetes, or other neuropathic conditions.—Authors' Abstract

Pereira, J. H., Cowley, S. A., Gschmeissner, S. E., Bowden, R. E. M. and Turk, J. L. Denatured muscle grafts for nerve repair; an experimental model of nerve damage in leprosy. J. Bone Joint Surg. [Br.] **72-B** (1990) 874–880.

About 20% of patients with leprosy develop localized granulomatous lesions in peripheral nerves. We report experiments in guinea pigs in which freeze-thawed autogenous muscle grafts were used for the treatment of such mycobacterial granulomas. Granulomas were induced in guinea pig tibial nerves, and the animals were left for 7 to 100 days in order to assess maximal damage. The local area of nerve damage was then excised and the gap filled with denatured muscle grafts. Clinical assessment after periods up to 150 days showed good sensory and motor recovery which correlated well with the histological findings. The muscle graft technique may be of value for the treatment of chronic nerve lesions in selected cases of leprosy.—Authors' Abstract

Ponnighaus, I. M., Boerrigter, G., Fine, P. E. M., Ponnighaus, J. M. and Russell, J. Disabilities in leprosy patients ascertained in a total population survey in Karonga District, northern Malaŵi. Lepr. Rev. **61** (1990) 366–374.

This paper describes the pattern of disability among 1654 leprosy patients ascertained between 1973 and 1987 in Karonga District, northern Malaŵi. Approximately 20% of patients identified prior to 1980 had some disability at registration, but this percentage fell to approximately 10% with the introduction of total population surveys in the LEPRA Evaluation Project. The proportion of patients with disabilities at registration increased with age, was higher among males than females, was higher among borderline and lepromatous than tuberculoid patients, and was higher for passively than for actively detected patients. The risk of developing disabilities among patients without any disabilities at registration was approximately 5 per 1000 person years, and appeared to be slightly higher after the completion of treatment than during treatment.—Authors' Summary

Thappa, D. M., Kaur, S. and Sharma, V. K. Disability index of hands and feet of patients attending an urban leprosy clinic. Indian J. Lepr. **62** (1990) 328–337.

One hundred eighty-nine leprosy patients, including 20 from a leprosy colony, having disabilities and deformities, were graded by the WHO (1960) classification and their disability indices were calculated. Disabilities occurred more frequently in males and the disability index was significantly higher in those with longer duration of the disease and in multibacillary patients. The majority of the disabled patients (82.5%) were manual workers, but the highest disability index was observed in beggars. Irregularly treated and untreated patients had significantly higher disability indices (DI 2.40 and DI 1.40) than those taking regular treatment (DI 1.09). No correlation was found between severity of disability and occurrence of type 1 and type 2 reactions. Disabilities of the hands and feet occurred with equal frequency.—Authors' Abstract

Other Mycobacterial Diseases and Related Entities

Affronti, L. F., Porrello, V. and Gupta, S. Trace elements incorporated into the culture medium of *Mycobacterium tuberculosis* promote the presence of tuberculoprotein C in the preparation of purified protein derivatives. Microbios **63** (1990) 101–107.

Two purified protein derivatives (PPDs) were prepared from *Mycobacterium tuberculosis,* H37Ra. One of the PPDs was prepared from the culture filtrate of organisms grown on Porskauer-Beck medium to which trace elements had been added. The other PPD was prepared from the culture filtrate of organisms grown on the same medium but without trace elements, and was used as the control. Comparative skin reactions in sensitized rabbits showed that the PPD prepared from organisms grown in the presence of trace elements was less potent than the control. Since it has long been recognized that of the many tuberculoproteins present in PPD, the C protein (a 44- to 66-kDa protein) was always the least potent fraction when tested in equivalent concentrations in both serological and skin-test assays, the possibility existed that the PPD obtained from organisms grown in trace element medium had more of the C-protein complex than the control. Comparative studies of these two PPDs in terms of their chemical composition, skin-test responses, and electrophoretic profiles obtained by SDS-polyacrylamide gel electrophoresis provide support for this assumption.—Authors' Abstract

Amicosante, G., Franceschini, N., Segatore, B., Oratore, A., Fattorini, L., Orefici, G., Van Beeumen, J. and Frère, J.-M. Characterization of a β-lactamase produced in *Mycobacterium fortuitum* D316. Biochem. J. **271** (1990) 726–734.

A β-lactamase from *Mycobacterium fortuitum* D316 was purified and some physicochemical properties and substrate profile determined. On the basis of its *N*-terminal sequence and of its sensitivity to β-iodopenicillanate inactivation, the enzyme appeared to be a class A β-lactamase, but its substrate profile was quite unexpected, since nine cephalosporins were among the 11 best substrates. The enzyme also hydrolyzed ureidopenicillins and some so-called "β-lactamase-stable" cephalosporins.—Authors' Abstract

Badukshanova, N. M., Vlasov, G. S., Vorobyev, A. A., Krasnoproshina, L. I., Fadeeva, N. I. and Zinin, A. E. [Use of the microdot enzyme immunoassay for the determination of antibodies to *Mycobacterium tuberculosis.*] Jh. Mikrobiol. Epidermiol. Immunobiol. **10** (1990) 98–103. (in Russian)

The microdot enzyme immunoassay (EIA) has been used for the determination of antibodies to *Mycobacterium tuberculosis* protein fractions, crude antigenic preparations, PPD, and old tuberculin in tuberculosis patients and healthy persons. Purified protein fractions have been found to possess the highest sensitivity and specificity in microdot EIA. The determination of antibodies to these fractions has permitted the differentiation of persons infected with *M. tuberculosis* from healthy ones. The use of *M. tuberculosis* protein fractions permits the determination of IgA and IgG in the sera of tuberculosis patients.—Authors' English Abstract

Bahr, G. M., Shaaban, M. A., Gabriel, M., Al-Shimali, B., Siddiqui, Z., Chugh, T. D., Denath, F. M., Shahin, A., Behbehan, K., Chedid, L., Rook, G. A. W. and Stanford, J. L. Improved immunotherapy for pulmonary tuberculosis with *Mycobacterium vaccae.* Tubercle **71** (1990) 259–266.

We previously demonstrated that a single intradermal injection of 10^9 irradiation-killed *Mycobacterium vaccae,* given 1 month after starting chemotherapy, caused significant changes in responses to mycobacterial antigens. Among 38 patients with pulmonary tuberculosis, 29% had lymphocytes responding to common mycobacterial anti-

gens after the injection, compared with only 11% of 49 similar patients after an injection of saline ($p < 0.03$). To increase the proportion of responders to these antigens, six modifications of the potentially immunotherapeutic injection, randomized with injections of saline, have been assessed by biochemical, clinical, hematological, immunological and radiological criteria. Subsequent lymphocyte proliferation to mycobacterial antigens enabled the modifications to be ranked in order of efficacy. Tuberculin plus murabutide plus 10^9 irradiated *M. vaccae* (36% of 25), an autoclaved preparation of 10^9 *M. vaccae* (45% of 22), and 2×10^9 irradiated *M. vaccae* (75% of 12) were the most effective. Antibody responses in several IgG subclasses to mycobacteria, but not streptococci, were also significantly increased by the most effective modifications over the 8 weeks following injection. Detailed radiological study showed that use of the autoclaved bacilli was followed by a delay in clearing of consolidation, but by better closing of cavities than was found in the control group, suggesting enhanced, or altered, immunological activity around the lesions.—Authors' Summary

Balasubramanian, R., Sivasubramanian, S., Vijayan, V. K., Ramachandran, R., Jawahar, M. S., Paramasivan, C. N., Selvakumar, N. and Somasundaram, P. R. Five year results of a 3-month and two 5-month regimens for the treatment of sputum-positive pulmonary tuberculosis in South India. Tubercle **71** (1990) 253–258.

A controlled study of three short-course regimens was undertaken in South Indian patients with newly diagnosed, sputum-positive pulmonary tuberculosis. The patients were allocated at random to one of three regimens: a) Rifampin, streptomycin, isoniazid and pyrazinamide daily for 3 months (R3); b) the same regimen as above but followed by streptomycin, isoniazid and pyrazinamide twice-weekly for a further period of 2 months (R5); c) the same as R5 but without rifampin (Z5). A bacteriological relapse requiring treatment occurred by 5 years in 16.8% of 113 R3, 5.2% of 97 R5, and 20.0% of 115 Z5 patients with organisms sensitive to streptomycin and isoniazid initially. The differences in the relapse rates between the R3 and R5 regimens and the R5 and Z5 regimens were statistically significant ($p < 0.01$ for both). Considering patients with organisms initially resistant to streptomycin or isoniazid or both, 7 of 52 patients (4 R3, 2 R5, 1 Z5) had a bacteriological relapse requiring retreatment.—Authors' Summary

Blanchard, D. K., Michelini-Norris, M. B. and Djeu, J. Y. A rapid [^{3}H]glycerol radioassay for determination of monocyte-mediated growth inhibition of *Mycobacterium avium*. J. Immunol. Meth. **133** (1990) 285–290.

[^{3}H]glycerol was used to radiolabel *Mycobacterium avium* (MA) bacteria after interaction with human monocytes in a rapid *in vitro* assay for determination of the growth inhibition of the mycobacteria by monocytes. Monocytes and MA were co-cultured in 96-well microtiter plates for 1–5 days, and [^{3}H]glycerol was added for an additional 3 days of incubation to radiolabel residual bacteria. The results indicate that monocytes inhibited mycobacterial growth within 24 hr of co-culture, an activity which increased during incubation until optimal growth inhibition was noted by 3–4 days. A comparison with conventional plate counting methodology demonstrated similar responses between the two assays except that the conventional assay required 2–3 weeks of culture before visible MA colonies could be detected and enumerated. Thus, the development of a rapid radiolabel assay to quantitate the interaction between monocytes and MA will facilitate the investigation of normal host responses to this opportunistic pathogen.—Authors' Abstract

Böddinghaus, B., Wolters, J., Heikens, W. and Böttger, E. C. Phylogenetic analysis and identification of different serovars of *Mycobacerium intracellulare* at the molecular level. FEMS Microbiol. Lett. **70** (1990) 197–204.

Comparative 16S rRNA sequencing was used to infer the phylogenetic relationship among different serovars of the *Mycobacterium avium–M. intracellulare* complex as

well as to define signature nucleotides characteristic for different serovars. In general, the groups defined by rRNA sequencing reflect the classification obtained with sensitin tests and pathogenicity examinations in chickens. Unique 16S rRNA sequence patterns could be defined for a) *M. avium,* b) *M. intracellulare* serovars 4, 5, 6, 8, 9, 10 and 11, c) *M. intracellulare* serovars 12, 13, 14, 15, 17, 19 and 20, d) *M. intracellulare* serovar 7 and e) *M. intracellulare* serovar 18. Phylogenetically, groups a and b on one hand and groups c, d and e on the other hand each share a common ancestor. *M. paratuberculosis* was indistinguishable from *M. intracellulare* serovars 4, 5, 6, 8, 9, 10 and 11 by this kind of analysis.—Authors' Summary

Crowle, A. J. and May, M. H. Inhibition of tubercle bacilli in cultured human macrophages by chloroquine used alone and in combination with streptomycin, isoniazid, pyrazinamide, and two metabolites of vitamin D_3. Antimicrob. Agents Chemother. **34** (1990) 2217–2222.

Intracellular tubercle bacilli (TB) reside in vacuoles in infected human macrophages (MPs). The relative impotency of streptomycin against TB in MPs and the contrary greatly increased potency of pyrazinamide (PZA) have been attributed to the fact that these vacuoles are phagolysosomes and, therefore, acidic. Chloroquine (CQ) is a lysosomotrophic base which can be used to raise phagolysosomal pH. Consequently, it was tested for its ability to increase the anti-TB effectiveness of streptomycin and decrease that of PZA in cultured human MPs. MPs infected with virulent Erdman strain TB were incubated in medium with various combinations of the drugs. Samples were taken at 0, 4, and 7 days and lysed for CFU counts of viable TB on nutrient agar. As expected, CQ increased the effectiveness of SM, but unexpectedly, it did not decrease that of PZA. CQ alone was found to be able to inhibit intracellular TB. Because of this, it was also tested with isoniazid, 1, 25$(OH)_2$-vitamin D_3, and 25-OH-vitamin D_3. It significantly enhanced the anti-TB protectiveness of both isoniazid and 25-OH-vitamin D_3. Some combinations of CQ and the various drugs tested were able to kill intracellular TB. These results suggest that CQ may be useful in the treatment of tuberculosis.—Authors' Abstract

Dickinson, J. M. and Mitchison, D. A. *In vitro* activities against mycobacteria of two long-acting rifamycins, FCE22807 and CGP40/469A (SPA-S-565). Tubercle **71** (1990) 109–115.

The *in vitro* activities of two new long-acting rifamycins, FCE22807 a derivative of FCE22250, and CGP40/469A (SPA-S-565), were studied. When compared with rifampin, the minimal inhibitory concentrations (MIC) against *Mycobacterium tuberculosis* of both were four times lower but neither was particularly active against rifampin-resistant strains of *M. tuberculosis* nor against *M. avium-intracellulare-scrofulaceum* complex strains. A drug is likely to be particularly effective in widely spaced intermittent dosage if it has a long half-life and high bactericidal activity. When tested against *M. tuberculosis* in the logarithmic and in the stationary phase of growth, FCE22807 was among the most bactericidal of the rifamycins, while CGP40/469A had little bactericidal activity.—Authors' Summary

Dovgalyuk, I. F., Semilutskaya, I. B., Tselikova, V. A. and Danilevskaya, I. M. [Clinical importance of HLA phenotype in childhood tuberculosis.] Probl. Tuberk. **9** (1990) 9–10. (in Russian)

According to HLA system (locuses A, B, C, DR), examination findings of 171 children aged between 1 and 15 years with different clinical manifestations of primary tuberculosis (including 49 with a pulmonary type of tuberculosis in different stages of inflammation, 46 with osteoarticular lesions of tuberculous etiology, and 76 with generalized forms of tuberculosis) indicate a significant rise in the incidence of HLA-B7, B14 antigens. Moreover, the incidence of HLA-B14 antigen increases sharply as the degree of clinical manifestations and severity of inflammatory changes become more evident. In intrathoracic adenopathy, the presence of HLA-DR, DR2 antigens seems to promote earlier clinicoroentgenologic manifestations and a more serious

course of an inflammatory process. In combined osteoarticular processes of specific etiology, an increase in the incidence of HLA-DR5, DR7 antigens is significant. The most evident drop in the immune T-system indices is traced in subjects bearing HLA-DR2 antigen. The given fact makes it possible to proceed on the assumption that genetically controlled immune response factors play a certain role in the pathogenesis of tuberculosis.—Authors' English Abstract

Gilleron, M., Venisse, A., Rivière, M., Servin, P. and Puzo, G. Carbohydrate epitope structural elucidation of ^{1}H-NMR spectroscopy of a new *Mycobacterium kansasii* phenolic glycolipid antigen. J. Biochem. **193** (1990) 449–457.

The complete primary structure of the carbohydrate moiety of a new phenolic glycolipid antigen, namely, PheGl K-IV from *Mycobacterium kansasii,* was successfully established from only one- and two-dimensional ^{1}H-NMR data. Among the scalar two-dimensional techniques, correlated spectroscopy with a 45° mixing pulse and phase-sensitive double-quantum-filtered correlated spectroscopy were selected, combined with two-dimensional dipolar techniques (nuclear Overhauser effect). These techniques using milligram of quantities native PheGl K-IV allowed the following monoacetylated tetrasaccharide to be proposed for its carbohydrate part: 4-*O*-Me-α-Man*p*-(1→3)-4-*O*-Ac-2-*O*-Me-α-Fuc*p*-(1→3)-2-*O*-Me-α-Rha*p*-(1→3)-2, 4-di-*O*-Me-α-Rha*p*. The PheGl K-IV shares, with the other phenolic glycolipids isolated from *M. kansasii* (K-I, K-II), a common core assigned to the lipid aglycone glycosylated by the monoacetylated trisaccharide part. It differs in the structure of the distal monosaccharide residue.—Authors' Abstract

Ginda, S. S. [Relation of sensitization to specific and nonspecific antigens to an individual type of immune reaction in pulmonary tuberculosis patients.] Probl. Tuberk. **9** (1990) 58–60. (in Russian)

Study of cellular sensitization and antibody formation in 317 patients with pulmonary tuberculosis (both concurrent and not concurrent with nonspecific lung diseases) to antigens of *Mycobacterium tuberculosis,* staphylococcus, streptococcus and pneumococcus revealed their various interaction in relation to the type of immune reaction. It was noted that with a normergic type of immune reaction, the levels of cellular sensitization and antibody formation to *M. tuberculosis* antigens were consistent with a hyperergic type, a higher level of cellular sensitization inhibited antibody formation; and with a hypoergic type, the inhibition of cellular sensitization level was accompanied by hyperproduction of antibodies. With an anergic type of immune reaction, inhibition of cellular sensitization and antibody formation to *M. tuberculosis* antigens was recorded.—Author's English Abstract

Grange, J. M. and Laszlo, A. Serodiagnostic tests for tuberculosis: a need for assessment of their operational predictive accuracy and acceptability. Bull. WHO **68** (1990) 571–576.

There have been numerous unsuccessful attempts to develop clinically useful serodiagnostic tests for tuberculosis. Although the large number of published reports clearly show that antibody levels are significantly higher in patients, as a group, than in a control population, little consideration is given to the value of the tests in various operational situations. In this paper we review the criteria generally used to assess the usefulness of a diagnostic test and introduce two new concepts—namely, operational predictive accuracy and operational acceptability.—Authors' Abstract

Jones, W. D., Jr. Geographic distribution of phage types among cultures of *Mycobacterium tuberculosis.* II. Cultures from India and South Africa. Am. Rev. Respir. Dis. **142** (1990) 1000–1003.

Mycobacterium tuberculosis cultures obtained from India and South Africa were phage-typed to determine distribution patterns according to phage type in these two geographic locations. Of the 74 Indian strains, 14.9% were type 1, 32.4% were type 2, 28.4 were type 7, and 24.3% were type 8; whereas of the 78 South African strains, 20.5% were type 1, 47.5% were type 2, 1.3%

were type 4, 11.5% were type 7, 19.2% were type 8. The phage types were then subdivided according to the lytic patterns produced by the six auxiliary phages. The phage-type distribution in the Indian and South African strains was compared with the type distribution in cultures from the United States and Southeast Asia, and differences were found in the four widely separated geographic areas.—Authors' Summary

Kelly, P., Burnham, G. and Radford, C. HIV seropositivity and tuberculosis in a rural Malawi hospital. Trans. R. Soc. Trop. Med. Hyg. **84** (1990) 725–727.

This study was undertaken to determine the extent to which human immunodeficiency virus (HIV) infection has increased hospital admissions for tuberculosis (TB) in a rural population of southern Malawi. The notes and chest X-radiographs of TB patients admitted to Malamulo hospital in 1983 and 1984, before the recognition of acquired immune deficiency syndrome (AIDS) in Malawi, were compared with those of patients admitted in 1987 and 1988. [The authors] found a 160% increase in TB admissions between the 2 periods. Extrapulmonary TB, especially pleural TB, was much commoner in 1987–1988 and occurred in a younger age group. HIV seroreactivity was measured in a third group of 152 tuberculosis patients admitted during 1988–1989. HIV seropositivity was found in 52% of all tuberculosis admissions and in 75% of those with extrapulmonary disease. There was no difference in clinical response to TB therapy between the HIV seropositive patients and those who were seronegative. Extrapulmonary TB should be considered in all HIV seropositive patients, especially in areas where the prevalence of TB is high. Healthy personnel involved in TB programs where HIV and TB infections are prevalent should plan for a large increase in the TB case load secondary to the HIV pandemic.—Authors' Abstract

McBride, M. E., Rudolph, A. H., Tschen, J. A., Cernoch, P., Davis, J., Brown, B. A., and Wallace, R. J., Jr. Diagnostic and therapeutic considerations for cutaneous *Mycobacterium haemophilum* infections. (Letter) Arch. Dermatol. **127** (1991) 276–277.

Among the nontuberculous species of *Mycobacterium* isolated from cutaneous lesions. *M. haemophilum* has received the least attention. Since first described in 1978, fewer than 20 cases have been reported worldwide. Clinical disease is usually associated with an underlying condition resulting in immunosuppression, and is characterized by cutaneous nodules or plaques on the extremities. The unique features that separate *M. haemophilum* from other *Mycobacterium* are the requirement of hemin for growth, and an unusual antimicrobial susceptibility pattern. We report a case in a nonimmunosuppressed host where the organism was isolated on routine bacteriologic medium and the patient was successfully treated with ciprofloxacin.—From the Letter

Perrone, C., Gikas, A., Truffot-Pernot, C., Grosset, J., Pocidalo, J.-J. and Vilde, J.-L. Activities of clarithromycin, sulfisoxazole, and rifabutin against *Mycobacterium avium* complex multiplication within human macrophages. Antimicrob. Agents Chemother. **34** (1990) 1508–1511.

The activities of clarithromycin, sulfisoxazole, and rifabutin against three virulent strains of *Mycobacterium avium* complex isolated from patients with acquired immunodeficiency syndrome were evaluated in a model of intracellular infection. Human monocyte-derived macrophages were infected at day 6 of culture with *M. avium* complex. Intracellular bacteria were counted 60 min after inoculation. Extra- and intracellular bacteria were counted at days 4 and 7 after inoculation. The concentrations used were 4 μg of clarithromycin per ml (MICs for the three strains, 4, 4, and 4 μg/ml), 50 μg of sulfisoxazole per ml (MICs, 50, 25, and 25 μg/ml), and 0.5 μg of rifabutin per ml (MICs, 2, 0.5, and 0.5 μg/ml). Compared with controls, clarithromycin and rifabutin slowed the intracellular replication of the three strains (at day 7 after inoculation, p was < 0.01 for the first strain and < 0.001 for the two others). Sulfisoxazole was ineffective against the three strains. Clarithromycin was as effective as rifabutin.

Clarithromycin plus rifabutin was as effective as each single agent. Clarithromycin plus sulfisoxazole was as effective as clarithromycin alone.—Authors' Abstract

Rakhimov, A. K. and Pospelov, L. E. [Study of genetic markers in the families of patients with tuberculosis.] Probl. Tuberk. **9** (1990) 7–8. (in Russian)

The article deals with the findings of the study of HLA-genotype influence on tuberculosis susceptibility in 26 families with tuberculosis patients. It was found that sensitivity to tuberculosis in the examined families is associated with HLA-DR2 antigen. Segregation analyses conducted in the families of patients with tuberculosis revealed a correlation between the sensitivity to tuberculosis and inheritance of certain HLA haplotypes from the affected parents to their children with tuberculosis.—Authors' English Abstract

Rastogi, N. and Blom-Potar, M.-C. A comparative study on the activation of J-774 macrophage-like cells by gamma-interferon, 1, 25-dihydroxyvitamin D_3 and lipopeptide RP-56142: ability to kill intracellularly multiplying *Mycobacterium tuberculosis* and *Mycobacterium avium.* Zentralbl. Bakteriol. **273** (1990) 344–361.

The J-774 macrophage-like cell line has been established as a model for intracellular multiplication of pathogenic mycobacteria, permitting assessment of the intracellular bactericidal action of the macrophages after addition of both the drugs and immunomodulators. In this study, the action of immunomodulators was investigated. Significant morphological changes were demonstrated under the optical and scanning electron microscope and the degree of macrophage activation was also measured by acid phosphatase (AcPase) cytochemistry, release of free oxygen radicals and by their ability to hinder the intracellular multiplication of virulent strains of *Mycobacterium tuberculosis (M.tb)* and *Mycobacterium avium (M.av).* For this purpose, the macrophages were left to multiply during 3 days in the presence of 50 U/ml of recombinant murine gamma-interferon (INF), 4 μg/ml of 1, 25 dihydroxyvitamin D_3 (D_3) and 50 μg/ml of lipopeptide RP-56142 (RP) added separately or in various possible combinations, and these "activated" cells were then challenged with viable bacteria. Parallel controls include bacterial multiplication in monoactivated macrophages and also extracellularly but under the same experimental conditions as in the macrophage experiments. Transmission electron microscopy using the AcPase marker to localize phagosome-lysosome fusion in infected cells was also performed. Although all the immunomodulators used significantly changed the morphology of treated cells and increased the percentage of AcPase-positive cells, none had any effect on the release of oxygen radicals. On the other hand, guinea pig alveolar macrophages which served as a parallel positive control were activated by INF and D_3 (but not RP) to release superoxide anions. Our data suggest that differential killing mechanisms for intracellular *M.tb* and *M.av* may exist. The results obtained also showed that established mycobactericidal mechanisms of the host could not solely account for the antimycobacterial effects observed. Consequently, mechanisms not yet revealed may account for some of the antimycobacterial effects observed.—Authors' Abstract

Rastogi, N. and Blom-Potar, M. C. Intracellular bactericidal activity of ciprofloxacin and ofloxacin against *Mycobacterium tuberculosis* H37Rv multiplying in the J-774 macrophage cell line. Zentralbl. Bakteriol. **273** (1990) 195–199.

Among new fluoroquinolone derivatives, the *N*-cyclopropylanalogs ciprofloxacin (CIPRO) and ofloxacin (OFLO) have recently been found to have a high *in vitro* activity against a variety of mycobacteria. In this study, we have investigated their action against intracellularly growing *Mycobacterium tuberculosis.* For this purpose, the J-774 macrophage cell line was infected with the H37Rv-type strain of *M. tuberculosis* and drugs at their serum level concentrations obtainable in healthy individuals (= 5 μg/ml) were added after 2 days of intracellular growth of the bacteria. The bacterial growth during the experimentation was followed both electron microscopically and also

by lysing the macrophages at various time intervals, and enumerating the bacterial viable counts after plating the lysate on appropriate media. Both drugs at the concentrations used did not affect macrophage viability (as assessed by trypan blue staining), and were highly bactericidal against the virulent tubercle bacilli multiplying in our J-774 macrophage model.—Authors' Abstract

Rastogi, N. and Goh, K. S. Action of 1-isonicotinyl-2-palmitoyl hydrazine against the *Mycobacterium avium* complex and enhancement of its activity by *m*-fluorophenylalanine. Antimicrob. Agents Chemother. **34** (1990) 2061–2064.

In the present work, we investigated whether resistance to isoniazid (INH) of organisms belonging to the *Mycobacterium avium* complex was caused by the bacterial cell envelope, with the cell wall and the outer layer acting as an exclusion barrier. We observed that this exclusion barrier was most efficient in excluding the hydrophilic drug INH, since this drug could not penetrate a wall matrix formed of various polymethylated lipidic or amphipathic substances. Two main strategies were proposed for circumventing this drug resistance: a) synthesis of amphipathic derivatives of otherwise highly hydrophilic drugs and b) inhibition of synthesis of the bacterial outer layer. The purpose of this work was to demonstrate that attaching a palmitic acid side chain to INH rendered it growth inhibitory against *M. avium* complex bacteria and that the concomitant use of this amphipathic INH derivative with *m*-fluorophenylalanine (an inhibitor of mycoside C biosynthesis which causes the disruption of the bacterial outer layer) resulted in further enhancement of its activity, leading to a bactericidal effect.—Authors' Abstract

Resnick, M., Roguel-Resnick, N., Bercovier, H., Levy, L., Toledo, J. and Zipori, D. Detection of interleukin-3 in the serum of mice infected with *Mycobacterium lepraemurium.* J. Infect. Dis. **162** (1990) 1202–1204.

Infection of mice by *Mycobacterium lepraemurium* is accompanied by ablation of erythropoiesis in the bone marrow and gross enlargement of the spleen. This, together with increased monocytopoiesis and the earlier demonstration of macrophage colony-stimulating factor in the serum of infected mice, suggested the activity of additional cytokines. Eight weeks after infection of mice by *M. lepraemurium,* interleukin-3 (IL-3) activity was demonstrated in the serum (titer, 1:3200). The serum titer of IL-3 activity was maximal after 13 weeks (> 1:6400) and was slightly reduced after 18 weeks (1:6400). That the IL-3 activity detected in the serum of the *M. lepraemurium*-infected mice reflected the presence of IL-3 itself was confirmed by a neutralization assay using anti-murine IL-3 antibodies; IL-3 activity in the serum of mice 13 weeks after infection was completely abolished by the anti-IL-3 antibodies. Finally, a 1-kb signal of IL-3 RNA was detected in the spleens of *M. lepraemurium*-infected mice 13 weeks after infection.—Authors' Abstract

Salem, J. I., Marója, J. de F., De Carvalho, F. F., de Lima, M. O. and Feuillet, A. Mycobacteria other than tubercle bacilli in sputum specimens from patients in Manaus (Amazonia, Brazil). Acta Amaz. **19** (1989) 349–354.

Sputum specimens were taken from 516 individuals in the state of Amazonas with clinical and/or radiological suggestion of pulmonary tuberculosis (144 had a previous history of the disease). By conventional mycobacteriological methods, *Mycobacterium tuberculosis* was isolated alone from 122 specimens and together with mycobacteria other than tubercle bacilli (MOTT) from 3 specimens; 139 isolates of MOTT were obtained. The most commonly isolated MOTT were *M. avium-intracellulare* and *M. fortuitum* (accounting for 7.36% and 6.20% of all sputum specimens, respectively). The authors draw attention to the high proportion of sputum samples containing MOTT (26.94%) in this region compared with values for isolates from France (3.74%) and elsewhere in Brazil (6.5%); they suggest that the source of the MOTT is the environment and that sensitization to environmental mycobacteria should be investigated locally in view of its possible interaction with vacci-

nation programs against leprosy and tuberculosis.—C. Brown (Trop. Dis. Bull.)

Sharma, V. K. Tuberculostatic activity of henna (*Lawsonia inermis* Linn.). Tubercle **71** (1990) 293–295.

The tuberculostatic activity of the herb henna (*Lawsonia inermis* Linn.) was tested *in vitro* and *in vivo*. On Lowenstein-Jensen medium, the growth of tubercle bacilli from sputum and of *Mycobacterium tuberculosis* H37Rv was inhibited by 6 μg/ml of the herb. *In vivo* studies on guinea pigs and mice showed that the herb at a dose of 5 mg/kg body weight led to significant resolution of experimental tuberculosis following infection with *M. tuberculosis* H37Rv.—Author's Summary

Stavri, D., Niculescu, D. and Stavri, H. Mycobacterial antigens and anti-idiotype antibodies revealed by an enzyme-linked immunosorbent assay in tuberculous patient sera. Rev. Roum. Biochim. **27** (1990) 3–11.

The mycobacterial antigens in tuberculous patient sera and in sera fractions obtained by gel permeation chromatography on Sephadex G200 column were investigated by an antigen-capture immunoassay. The antigens were found either free or combined with specific immunoglobulins as immune complexes. The method seems to reveal also the immunoglobulins which mimic the mycobacterial antigen structure, respectively the anti-idiotype antibodies ($Ab_2\beta$), which appear spontaneously as part of the natural immune response in some tuberculous patient sera.—Authors' Abstract

Yajko, D. M., Sanders, C. A., Nassos, P. S. and Hadley, W. K. *In vitro* susceptibility of *Mycobacterium avium* complex to the new fluoroquinolone sparfloxacin (CI-978; AT-4140) and comparison with ciprofloxacin. Antimicrob. Agents Chemother. **34** (1990) 2442–2444.

We tested the activity of the new fluoroquinolone sparfloxacin (CI-978; AT-4140) against 30 strains of *Mycobacterium avium* complex (MAC) isolated from patients with acquired immune deficiency syndrome. MICs of sparfloxacin (range, ≤ 0.06 to 4 μg/ml) were lower than MICs of ciprofloxacin for all 30 strains, and MBCs for acid-fast bacteria were lower for 28 of the 30 strains. In synergism experiments using 10 strains of MAC, fractional inhibitory concentration indices revealed that the combination of sparfloxacin plus ethambutol was synergistic against 9 strains, and the three-drug combination of sparfloxacin plus ethambutol plus rifampin was synergistic against all strains. In the absence of ethambutol, the combination of sparfloxacin plus rifampin appeared to be antagonistic against three of the MAC strains.—Authors' Abstract