

CURRENT LITERATURE

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

General and Historical

McDougall, A. C. and Georgiev, G. D. Priorities in leprosy control. (Editorial) *Lepr. Rev.* **60** (1989) 1-7.

The editorialists discuss priorities in leprosy control under the headings of "strategic priorities" and "operational priorities." The former include: implementation of multi-drug therapy (MDT), acceptance of WHO recommended rather than other MDT regimens, integration of MDT with primary health care, training of medical and health staff, and operational research. The latter include: management, training of health staff at district level, and new strategies and technology. The emphasis throughout is on case-detection and MDT. The article closes with two questions: "Why is it that so much money and effort is put into laboratory-based research, and so little into the study of the operational aspects of this disease, for which treatment is available and effective? Should we try harder to define and agree on the priorities so that available manpower, money and effort are used to the greatest possible advantage?"—C. A. Brown (*Trop. Dis. Bull.*)

Noordeen, S. K. Technology for leprosy control—progress and prospects. (Kellersberger Memorial Lecture, 1988). *Ethiop. Med. J.* **27** (1989) 161-180.

Looking at the future, one should recognize that we do not know enough about leprosy. There are many gaps in the knowledge on the epidemiology of leprosy. It is difficult to work with *Mycobacterium leprae* as it could not be cultivated *in vitro* so far. Several areas of immunology of leprosy are still unclear despite the progress made in recent

years. It is only very recently that we have found a truly effective chemotherapy against the disease, even though it cannot be considered as ideal. Immunopathogenesis of complications, such as reactions and neuritis, are not fully understood and neither do we have effective means of preventing or curing them. We have as yet no prophylactic method to prevent the disease. No doubt progress is being made in research in all the above areas. Notwithstanding the above, the available knowledge, technology and methods developed mostly during the past few decades are indeed formidable and capable of bringing about major reductions in leprosy. In this connection the most important and critical technology to be mentioned is MDT. Provided clear national commitments are made to deal effectively with leprosy, provided adequate resources are made available, and provided the health infrastructure is reasonably adequate, MDT can reduce the global leprosy case-load by about 75% in the next 5 to 10 years. This in itself could be considered an enormous achievement.

However, the remaining leprosy problems in the later phases of MDT are not going to be easy to solve, as they will consist of the breaking down of old subclinical infections, difficult-to-reach cases, chronic defaulters and possibly some relapses, all occurring in a background of very low endemicity. This stage, which could emerge around the turn of the century, will warrant additional tools and additional strategies to deal with the remaining problems. It is hoped that the ongoing research efforts, including those on vaccines, would provide the necessary means to deal with the problems.—(From the Lecture)

Chemotherapy

Cestari, T., Nagel, R. and Fontoura Heidrich, R. [Lichenoid eruption due to dapsone in a leprosy patient.] *An. Bras. Dermatol.* **64** (1989) 227–229. (in Portuguese)

The authors present a case of leprosy with lichenoid eruption after a 30-day treatment with dapsone confirmed later by the re-administration of the drug. The side effects caused by dapsone and the low frequency of this kind of drug eruption are pointed out.—Authors' English Summary

Chen, J.-K., Wang, S.-Y., Hou, Y.-H., Ni, G.-X., Zhang, J.-L. and Tang, Q.-G. Primary dapsone resistance in China. *Lepr. Rev.* **60** (1989) 263–266.

Ninety-seven strains of *Mycobacterium leprae* recovered from patients with previously untreated multibacillary leprosy were tested for dapsone susceptibility. The specimens originated from Shanghai Municipality, Jiang-su Province and Fu-jian Province. Approximately 28% of the strains either did not infect the mice or the results of susceptibility were inconclusive due to the low proportion of viable organisms in the bacterial populations. Among the 70 strains in which dapsone susceptibility could be tested in mice, 31 (44%) strains were found with primary dapsone resistance. Although the majority of the primary dapsone-resistant strains were shown to be of a low- or intermediate-degree, one sixth of them were of high-degree resistance.—Authors' Summary

Coleman, M. D., Breckenridge, A. M. and Park, L. K. Bioactivation of dapsone to a cytotoxic metabolite by human hepatic microsomal enzymes. *Br. J. Clin. Pharm.* **28** (1989) 389–395.

Using human mononuclear leukocytes as target cells, we have investigated the bioactivation of dapsone (DDS) to a cytotoxic metabolite in the presence of microsomes from nine human livers. Values for NADPH-dependent toxicity ranged from 8.8–27% ($15.8 \pm 5.9\%$) and were similar to those for microsomes from control mice, 16–24% ($19.0 \pm 4.8\%$). Microsomes pre-

pared from mice induced with either phenobarbitone or beta-naphthoflavone did not produce significantly more NADPH-dependent toxicity than microsomes prepared from control mice. Cytotoxicity was abolished not only by ascorbic acid, but also by sub-physiological concentrations of *N*-acetylcysteine and glutathione. DDS was metabolized *in vitro* to a hydroxylamine (metabolic conversion $3.1 \pm 1.5\%$), which was oxidized further to a cytotoxic metabolite which also became irreversibly bound to protein.—Authors' Abstract

da Costa Rocha, A. L., Lopes dos Santos, M. A., Garcia da Veiga, R. R. and Pereira, A. C., Jr. [Sulfone—pharmacological aspects, new indications besides hanseniasis, drug resistance.] *An. Bras. Dermatol.* **64** (1989) 235–238. (in Portuguese)

The authors comment about the pharmacologic aspects of 4', 4' diaminodiphenylsulfone, showing the most important pathologies which have been improved by the use of the drug and direct a person's attention to the risk of its indiscriminate use as a factor that would propitiate sulfone resistance in endemic areas of Hansen's disease. Because they consider it an important problem, they comment about sulfone resistance.—Authors' English Summary

Figueiredo, M. S., Pinto, B. O. and Zago, M. A. Dapsone-induced haemolytic anaemia and agranulocytosis in a patient with normal glucose-6-phosphate-dehydrogenase activity. *Acta Haematol. (Basel)* **82** (1989) 144–145.

A case of dapsone-induced severe hemolytic anemia and agranulocytosis is described. A possible common pathogenic mechanism for the simultaneous occurrence of these side effects of dapsone therapy in a patient with normal glucose-6-phosphate-dehydrogenase activity is proposed.—Authors' Abstract

Franzblau, S. C., White, K. E. and O'Sullivan, J. F. Structure-activity relationships of tetramethylpiperidine-substituted phenazines against *Mycobacterium*

leprae in vitro. Antimicrob. Agents Chemother. **33** (1989) 2004–2005.

In a previous study of structure-activity relationships of selected phenazines against *Mycobacterium leprae* in vitro, compounds containing a 2,2,6,6-tetramethylpiperidine substitution at the imino nitrogen were most active. Therefore, the effect of substitution at the *para* positions of the phenyl and anilino groups in tetramethylpiperidine-substituted phenazines was assessed. As determined by radiorespirometry, activity in ascending order was observed in compounds substituted with hydrogens or fluorines, ethoxy groups, methyl groups, chlorines, and bromines and correlated with partition coefficients in octanol-water.—Authors' Abstract

Gu, C., et al. [Histological chemistry study and quantitative measurement on skin pigmentation of B663 in leprosy.] Chin. J. Clin. Dermatol. **18** (1989) 64–66. (in Chinese)

The skin pigmentation of multibacillary leprosy patients after taking clofazimine (B663) for 3 weeks or 3 months was studied by histological chemistry and quantitative measurement of B663. Small round particles and B663 crystals were found in sections of patients having been treated for 3 weeks, mainly deposited in the reticulo-endothelial cells and fat tissues. In three cases, there was brown pigment in sections of patients on treatment for 3 months. The average quantity of B663 was 13.528 $\mu\text{g/g}$ in active lesions, while it was only 2.876 $\mu\text{g/g}$ in nonactive skin lesions. The relations between the degree of B663 pigmentation, the types of leprosy, cellular infiltrations, and bacilli are discussed in this article.—From Authors' English Abstract

Haas, M. and Harrison, J. H., Jr. Stimulation of K-Cl cotransport in rat red cells by a hemolytic anemia-producing metabolite of dapsone. Am. J. Physiol. **256** (Cell Physiol. **25**) (1989) C265–C272.

Dapsone, a sulfone compound used in the treatment of leprosy and, more recently, *Pneumocystis carinii* pneumonia, produces as a major side effect a hemolytic anemia. This anemia is characterized by oxidation

of hemoglobin to methemoglobin and increased splenic uptake of red blood cells. Using a rat model, Grossman and Jollow found that dapsone hydroxylamine (DDS-NOH), a dapsone metabolite, is responsible for its hemolytic effect *in vivo*. DDS-NOH also promotes hemoglobin binding to SH groups on rat red cell membrane proteins. Since the binding of hemoglobin and other reagents (e.g., *N*-ethylmaleimide) to membrane SH groups has been associated with increased K transport in red blood cells, we examined the effect of DDS-NOH on K efflux from rat red blood cells *in vitro*. Cells shrink when exposed to DDS-NOH (100 μM) in media with plasma-like ionic composition. This shrinkage is prevented if extracellular K is raised to 110 mM or if intra- and extracellular Cl are replaced by methylsulfate (MeSO_4), suggesting involvement of a K-Cl cotransport pathway. Indeed, 100 μM DDS-NOH produces a 4- to 5-fold increase in K efflux in cells containing Cl but less than a 2-fold increase in cells containing MeSO_4 . This stimulatory effect is specific for K; Na efflux is slightly inhibited by 100 μM DDS-NOH. The concentration of DDS-NOH required for half-maximal stimulation of Cl-dependent K efflux (53 μM) is similar to its half-maximal hemolytic concentration in rats ($\sim 100 \mu\text{M}$). Furthermore, the stimulation of Cl-dependent K efflux by DDS-NOH is > 80% reversed by subsequent treatment of the cells with dithiothreitol, suggesting involvement of SH groups. Our results indicate that DDS-NOH exposure stimulates an apparent K-Cl cotransport in rat red blood cells, resulting in cell shrinkage under physiological ionic conditions. Since shrinkage of red blood cells renders them less deformable, this suggests a pathophysiological mechanism whereby DDS-NOH exposure *in vivo* could promote increased splenic uptake of red blood cells and hemolytic anemia.—Authors' Abstract

Iredale, J. P., Sankaran, R. and Wathen, C. G. Cutaneous vasculitis associated with rifampin therapy. Chest **96** (1989) 215–216.

Immune complex-mediated vasculitis is a well-recognized form of idiosyncratic drug reaction. We report cutaneous vasculitis in

association with therapy with rifampin. To our knowledge, this has not previously been documented. Rifampin is widely used, and such reactions are therefore important to note.—Authors' Abstract

Lin, C. [Clofazimine poisoning of the eighth cranial nerves in eight cases of leprosy.] *China Lepr. J.* **5** (1989) 80–81. (in Chinese)

Eight cases with clofazimine poisoning of the eighth cranial nerves 6–50 days after taking MDT were reported, out of which two cases are relatively serious, two moderate, and four slight. The symptoms of the poisoning includes dizziness, tinnitus and decreased hearing, and will disappear in 20–40 days after stopping the drug. When the poisoning symptoms are slight, the treatment may be continued.—Author's English Abstract

Paksoy, N. and Levi, V. Preliminary assessment of multidrug therapy in leprosy patients in Western Samoa. *N.Z. Med. J.* **102** (1989) 580–591.

Western Samoa is an island state in the Polynesian part of the South Pacific Ocean with a total population of around 160,000. In 5 July 1985 multidrug therapy (MDT), as advised by the WHO, was started in leprosy patients there. One hundred eighteen cases (99 males, 19 females) on the active list were included in the MDT program. Sixty-three (53.3%) were paucibacillary and 55 (46.6%) were multibacillary. Eighteen patients (15.26%, 15 paucibacillary, 3 multibacillary) were below 15 years of age. In this paper we present a preliminary evaluation of WHO/MDT in leprosy patients in Western Samoa as of 31 December 1987. Assessment was done on clinical, bacteriological and histopathological grounds. During the evaluation period the acceptance of MDT by Samoan patients was found adequate, even though surveillance is still continuing. As of 31 December 1987 there were 40 patients (34 male, 6 female) on the active list. Thirty-five (87.5%) were on the multibacillary and 5 (12.5%) on the paucibacillary regimen. Only 2 patients (both paucibacillary) were below 15 years of age. The prevalence rate was now estimated to be 0.25/1000. Leprosy cannot be considered a

serious public health problem in the country. However, there is still need for improvement in case-finding, recording, and follow up matters.—Authors' Abstract

Pritze, S., Alvarenga, A. E., Leguizamon, O. and Haubitz, I. Isoprodian and rifampicin in the treatment of leprosy: a descriptive evaluation of therapy durations in 475 Paraguayan leprosy patients. *Chemotherapy* **35** (1989) 373–382.

In Paraguay, the National Leprosy/Tuberculosis Program is based on a combined chemotherapy with isoprodian and rifampin. The aim of this descriptive study was to investigate the therapy durations used so far in the treatment of 475 leprosy patients and to analyze the criteria responsible for the wide-ranging differences in therapy durations. As initial criteria, the following parameters were identified to have a significant influence on the therapy duration: patients never treated before or pretreated, clinical classification and initial bacterial index (BI) value. During therapy, conditions like the attendance and BI decrease/year showed a significant correlation with the therapy duration. Even though the studied criteria did not allow us to draw a definite conclusion with regard to an "ideal" therapy duration, they proved to be reliable, since only two patients have relapsed so far.—Authors' Abstract

Quigley, J. M., Blake, J. M. and Bonner, F. J. The effect of ionization on the partitioning of clofazimine in the 2,2,4-trimethylpentane–water system. *Int. J. Pharm.* **54** (1989) 155–159.

The pH dependence of the 2,2,4-trimethylpentane–water partition coefficient of clofazimine is studied at 37°C. A model is presented which includes contributions from the ionized (P_i) and unionized (P_u) species to the observed apparent partition coefficient (P'). The value of P_i is much smaller than that of P_u with the ratio P_i/P_u , designated Q , equal to 1.012×10^{-4} . However, at low pH, the former term becomes increasingly predominant, and it is desirable to assess its contribution quantitatively

to the apparent partition coefficient.—Authors' Summary

Richardus, J. H. and Smith, T. C. Increased incidence in leprosy of hypersensitivity reactions to dapsone after introduction of multidrug therapy. *Lepr. Rev.* **60** (1989) 267–273.

In order to address the question whether hypersensitivity reactions to dapsone are becoming more frequent, the clinical data of 7300 leprosy patients treated between 1949 and September 1988 at the McKean Rehabilitation Centre in Thailand were reviewed. Information from the period 1949 to 1969 was too incomplete to allow conclusions. The incidence of hypersensitivity reactions to dapsone between 1970 and 1982 was 0.3%. From 1982 (with the introduction of multidrug therapy) to September 1988, the incidence was 3.6%, a tenfold increase compared with the previous period. Of the 19 cases seen since 1982, 12 were diagnosed as dapsone syndrome. Of a total of 13 patients seen since 1980 with dapsone syndrome, 3 ended fatally, indicating the severity of the complication. The question is raised whether an unexplained drug interaction with rifampin is responsible for the increase of hypersensitivity reactions to dapsone in patients treated for leprosy.—Authors' Summary

Roy, S., Das, S. K., Pal, M. and Gupta, B. K. Design and *in vitro* evaluation of dapsone-loaded micropellets of ethyl cellulose. *Pharm. Res.* **6** (1989) 945–948.

A controlled-release dosage form was manufactured by dispersing ethyl cellulose sol in acetone into a medium of mineral oil. Dapsone was used as the model drug. The powdered drug was dispersed in the ethyl cellulose sol, and the formulation variables affecting the production of the discrete and spherical micropellets and their size distribution were investigated. The percentage of SPAN 80 in the formulation affected the yield and physical properties of the micropellets. The *in vitro* drug release followed first-order diffusion-controlled dissolution. More than 85% of the drug was released over 5 hr for all formulation batches, with

delayed release over the drug dissolution profile.—Authors' Abstract

Self, T. H., Tsiu, S. J. and Fowler, J. W., Jr. Interaction of rifampin and glyburide. (Letter) *Chest* **96** (1989) 1443–1444.

Numerous clinically significant drug interactions have been reported with the use of rifampin, including the sulfonylureas, tolbutamide and chlorpropamide. We recently observed a case suggestive of an effect of rifampin on serum glyburide concentrations. To our knowledge, this is the first report of this interaction in the literature.—(From the Letter)

Suite, M. and Edinborough, N. B. A second report on multidrug therapy for leprosy in Trinidad and Tobago. *Lepr. Rev.* **60** (1989) 288–299.

Multidrug therapy consisting of rifampin, clofazimine and dapsone was introduced to Trinidad and Tobago in January 1982. This was with slight modification of the WHO regimens. Since then 717 patients have completed multidrug therapy up to the end of December 1987. Of these, 272 patients have completed surveillance and have been discharged from clinic attendance. Thirty-four patients died before completing surveillance and three are known to have migrated. Of the remaining 408 cases still under surveillance, the majority are multi-bacillary. This paper reviews the outcome of multidrug therapy in Trinidad and Tobago between January 1982 and December 1987, a period of 6 years, and presents some of the statistics related to the newly diagnosed patients within the same period.—Authors' Summary

Vaz, M., Jacob, A. J. W. and Rajendran, A. "Flu" syndrome on once monthly rifampicin: a case report. *Lepr. Rev.* **60** (1989) 300–302.

"Flu" syndrome as a complication of intermittent weekly administration of rifampin is well documented. The rare occurrence of "flu" syndrome on once monthly rifampin is reported in this paper.—Authors' Summary

White, N. W. Venous thrombosis and rifampicin. *Lancet* 2 (1989) 434–435.

In a retrospective analysis of clinically diagnosed and lower-limb deep-vein thrombosis (DVT) proven by contrast venography, DVT complicated admissions in 46 (3.4%) of 1366 adult patients treated in a tuberculosis hospital during 1986. Analysis of 7542 admissions during 1978–1986 showed a relative risk of 4.74 in patients

treated with regimens including rifampin compared with other regimens. DVT was significantly more common in winter months and usually occurred within 2 weeks of treatment being started. This probable association between rifampin and DVT does not contraindicate use of this drug, but measures to prevent DVT should be taken in inpatients receiving rifampin.—Author's Summary

Clinical Sciences

Ashmalla, L., Yang, S. J., Michel, M. and Rafla, L. T lymphocyte subsets in leprosy; a study of 24 Egyptian patients. *Int. J. Dermatol.* 28 (1989) 451–453.

Phenotypic analysis was done on 24 Egyptian leprosy patients and 11 healthy controls. The type of leprosy, duration of disease at the time of testing, and age were found to affect T-cell subset distribution. As compared with controls, neural leprosy tended to have a decreased total T-cell percentage, borderline leprosy an increased T-suppressor-cell percentage, and reactional borderline lepromatous leprosy an increased T-helper/suppressor ratio. Patients with the disease for less than 1 year had a higher mean percentage of T-suppressor cells and a lower mean T-helper/suppressor ratio than patients with leprosy for more than 1 year. The same was true in older (50–70 years old) versus younger (12–41 years old) patients.—Authors' Abstract

Chaudhury, S., Hazra, S. K., Dey, S. K. and Saha, B. Demonstration of acid fast bacilli in the circulating immune complex of leprosy cases and contacts. *Indian J. Dermatol.* 33 (1988) 52–55.

Although bacillema in leprosy is an established fact it has not yet been possible to demonstrate acid-fast bacilli (AFB) in a substantial number of cases. This is due to the fact that AFB remain bound in the serum as immune complex (IC). Dissociation of IC results in release of antigen (AFB). Following IC dissociation it has been possible to demonstrate AFB in 100% of lepromatous cases, 60% of tuberculoid cases, and

20% of contacts of active lepromatous cases.—Authors' Abstract

Deguerri, M., Declercq, E., Misson, C., Vellut, C. and Bertrand, F. Registration of the number of macules in paucibacillary leprosy for evaluation of early diagnosis and individual prognosis. *Lepr. Rev.* 60 (1989) 206–213.

The number of macules is usually registered at diagnosis in the first clinical examination of leprosy patients. The question studied here is whether this practice is of any interest as an indicator of the precocity of detection or the prognosis. The study is based on the 26,996 paucibacillary patients detected from 1957 to 1982 in Polambakkam Leprosy Centre (South India) for whom the number of macules and disability status are assessed and registered. Several observations suggest that the proportion of single-macule patients among the newly detected cases is a more sensitive indicator than the proportion of new patients with disabilities for the evaluation of the delay between onset of the disease and detection. Its use could be especially helpful for programs running for several years, when it becomes difficult to observe significant variations in the proportion of patients with disabilities. Regarding the prognosis value of the number of macules, inactivation and relapse probabilities were calculated. Regularity of treatment is found to be a better predictor of early inactivation than the number of macules, while relapse probabilities are more affected by the number of macules.—Authors' Summary

Duvauchelle, D. and Goldstein, N. Cauliflower growths in ulcers of leprosy—a question of malignancy. *Hawaii Med. J.* **48** (1989) 129–131.

Cauliflower growths arising in trophic ulcers of Hansen's disease patients are not necessarily malignant. These types of growths can also be seen as pseudoepithelioma, chronic granulomas, and pseudoepitheliomatous hyperplasia. A biopsy should always be taken to rule out the possibility of a malignancy, however. Should the diagnosis be pseudoepitheliomatous hyperplasia, a less aggressive mode of therapy may be tried at first, especially if an amputation will markedly alter the patient's quality of life. Nevertheless, for pseudoepitheliomatous hyperplasia, wide excision is the treatment of choice where feasible, depending on size, extent and involvement of deeper structures.—Authors' Summary

Huang, L., et al. [Preliminary study on antinerve antibody in the sera of leprosy patients.] *China Lepr. J.* **5** (1989) 7–10. (in Chinese)

The circular antinerve antibody was examined with indirect enzyme linked immunohistochemical assay in 36 cases of leprosy and in 10 normal controls. The result shows that the positivity is 83.33% in the leprosy patients. The enzyme-linked monoclonal antibody of the goat anti-human IgG is able to put color on the nerve tissue. In the sections of the nerve the antinerve antibody is mainly combined with the membranes of myelin sheath cells and the nerve axons. Weak positivity may be seen in the sera of 10 healthy controls, while the dilution titer is in 1:50 or 1:100. The authors consider this method could be used for the early diagnosis leprosy, to judge the degree of the nerve injury and to assess the prognosis of the patients as a reference index.—Authors' English Abstract

Lin, D., et al. [Survey of the first skin lesions in 343 cases of leprosy.] *China Lepr. J.* **5** (1989) 76–79. (in Chinese)

The survey of the first lesions of the skin in 434 leprosy patients shows they are anesthetic maculae in 186 cases, accounting for 42.8%; hypopigmented maculae in 83

(19%); erythema in 51 (11.1%); big tuberculoid plaques in 31 (13.4%) and some infiltrations or nodules and those occurred on the hands in 21.2% of the cases, on the legs in 18.7% and on the face, neck and extremities in 93.1%. The authors believe that an understanding of the location and type of the first skin lesion is helpful in the early diagnosis of leprosy.—Authors' English Abstract

Loretti, A. Leprosy control: the rationale of integration. *Lepr. Rev.* **60** (1989) 306–316.

After considering the situation and the perspectives of integration and the drawbacks that a vertical approach can represent for leprosy control, the author proposes the framework of control programs as a systemic model for comprehensive health care. The structure that health services in developing countries are adopting in order to implement PHC allows for an horizontal integration of specific activities; conversely, activities which have already proved their value for leprosy control can easily enlarge their scope and include other prevalent conditions. Integration leads to an improvement in patients' and health workers' attitudes; provided that the necessary supervision is guaranteed, integration is feasible and warrants more effective patient care and a better use of resources in order to reduce the specific risk in the community.—Author's Summary

McDougall, A. C. and Georgiev, G. D. Educational material for the patient with leprosy. *Lepr. Rev.* **60** (1989) 221–228.

This paper addresses the need for suitably written and illustrated material for the patient with leprosy with emphasis on the effective administration of WHO recommended multiple drug therapy (MDT) and the prevention of deformities. The successful implementation of MDT strategy for leprosy control calls for attention to a "package" of activities, among which the education of the patient regarding the disease and its modern treatment may be of crucial importance. Attention is drawn to the steady improvement in educational and literacy levels in many developing countries

and to the potential of clearly written instructions for use by patients and staff. The importance of development of educational material in the context of the regional and local cultural milieu is stressed. Eight major "messages" related to the causation of the disease, the importance of regular clinic attendance for monthly supervised drug administration, compliance to the daily domiciliary drug intake and action for prevention of disability, are proposed. These "messages" are accompanied by outline drawings which can be used by staff for patient education both at the onset of chemotherapy and on the subsequent clinic attendances. The paper describes broad approaches but underlines the importance of local development of educational material for this purpose.—Authors' Summary

Parikh, D. A., Parikh, A. C. and Ganapati, R. Penile and scrotal lesions in leprosy: case reports. *Lepr. Rev.* **60** (1989) 303–305.

Six leprosy patients in the Ridley-Jopling spectrum of BT-BL showing lesions on penis and scrotum are presented since we believe that this common-enough clinical feature is not well documented in the literature.—Authors' Summary

Patki, A. H. and Mehta, J. M. Dapsone-induced erythroderma with Beau's lines. *Lepr. Rev.* **60** (1989) 274–277.

A 35-year-old female with borderline lepromatous (BL) leprosy who suffered from dapsone-induced erythroderma is reported. Sudden onset of erythroderma gave rise to a temporary arrest of the function of nail matrix with the resultant Beau's lines. She rapidly recovered with omission of dapsone and therapy with systemic corticosteroids and a topical emollient. In view of the potentially fatal hypersensitivity reaction, we suggest that any patient on multidrug therapy for leprosy needs an urgent referral to a dermatologist if the patient develops a skin rash during the first 2 months of treatment.—Authors' Summary

Rabinowitz, L. G., Yuengel, C., Honig, P. J. and Solomon, A. R. A papulosquamous eruption in a 12-year-old Cambodian boy. *Pediatr. Dermatol.* **6** (1989) 152–154.

A 12-year-old Cambodian boy, in the United States since 1982, was examined in the dermatology clinic at Children's Hospital of Philadelphia with a 1-year history of an erythematous, scaly, pruritic eruption on his face, lips, and nose. The eruption waxed and waned. His general health was good and he offered no other complaints. The rash had been treated in the past with clotrimazole cream and penicillin with no improvement. Physical examination revealed annular lesions with erythematous, papular, scaly borders and central clearing. These lesions were distributed on the right cheek and across the nasal bridge, as well as on the hands, wrists, and extremities. A 10-cm arcuate lesion was present on the buttocks. There was hypesthesia to pinprick. The great auricular nerve was enlarged bilaterally. Results of complete blood cell count and chemistry panel were within normal limits. A skin biopsy specimen was obtained from his buttocks which showed borderline tuberculoid leprosy. The patient was initially treated with rifampin, 300 mg twice a day, alone because he was found to be glucose-6-phosphate dehydrogenase (G6PD) deficient. Because of the need for combination therapy, clofazimine, 50 mg daily, was added. He is currently doing well and all lesions have cleared.—(From the Report)

Ridley, M. J. The cellular exudate-*Mycobacterium leprae* relationship and the critical reading of skin smears. *Lepr. Rev.* **60** (1989) 229–240.

A careful reading of conventionally stained Ziehl-Neelsen skin-smear preparations in leprosy provides a number of insights into the patient's situation, including his approximate position in the spectrum. This data serves as a cross-check on the primary results of the smear examination, and aids their interpretation for the purposes of diagnosis, assessment of the response to chemotherapy and the possible onset of relapse.—Author's Summary

Saxena, N., Sharma, R. P. and Singh, V. S. Study of serum calcium and magnesium in leprosy. *Indian J. Dermatol.* **33** (1988) 48–51.

Serum calcium and magnesium levels were determined in 60 patients suffering

from different types of leprosy. These findings were evaluated in comparison to 20 normal subjects serving as controls. Serum calcium levels were significantly decreased in lepromatous leprosy (6.9 ± 0.9 mg/dl $p < 0.001$). The decrease in serum calcium levels was related with the severity and the duration of the leprosy lesions. The serum calcium levels in borderline and tuberculoid forms were also low but were not statistically significant. A significant decrease in serum magnesium levels was observed in all types of leprosy. Lepromatous leprosy patients showed an highly significant decrease in serum magnesium levels (1.10 ± 0.18 mg/dl $p < 0.001$).—Authors' Abstract

Sehgal, V. N. Inoculation leprosy; current status. *Int. J. Dermatol.* **27** (1988) 6–9.

There is overwhelming evidence to indicate that transmission of leprosy may occur through inoculation. Should this aspect be incriminated in TT and BT occupying the exposed part of the extremities or those parts of the body amenable to trauma or injury, we may be able to define more cases of this entity. The importance of asepsis and sterilization should be emphasized to tattooers and vaccinators. The preceding measures will be of immense value in case detection, further underlining the importance of the entity and its ultimate treatment and prevention.—Author's Conclusions

Sehgal, V. N. and Joginder. Leprosy in children: correlation of clinical, histopathological, bacteriological and immunological parameters. *Lepr. Rev.* **60** (1989) 202–205.

The study of leprosy in children has indicated an incidence of 10% among leprosy patients attending the clinic. The duration of the disease was usually less than 2 years. The expression of leprosy in this group was either a macule and/or a plaque. Classification was, therefore, different and conforms to indeterminate (I), borderline tuberculoid (BT), and borderline (BB) leprosy. Only occasionally were other clinical variants seen. The bacteriology was largely unproductive by slit-skin smears. The lepromin (Mitsuda) responses were positive in BT and unpredictable in BB patients. Epicutaneous responses to sensitization with

dinitrochlorobenzene (DNCB) paralleled responses to lepromin. Microscopic pathology was of very little help. The correlation of these parameters was only 50%–60%, indicating that the diagnosis of leprosy should primarily be based on clinical features.—Authors' Summary

Srinivasan, H. Do we need trials of agents alleged to improve healing of plantar ulcers? *Lepr. Rev.* **60** (1989) 278–282.

The assumptions underlying trials of agents claiming to heal plantar ulcers “faster” and “better” are shown to be fallacious, and it is pointed out that in most cases these ulcers fail to heal for lack of attention and not for want of a specific topical agent. Clinical trials in this area are difficult and are not worth the trouble since they do not add to our knowledge about these ulcers or their management in the clinic or in the field.—Author's Summary

Srinivasan, H. and Stumpe, B. Value of thermal sensibility testing in the field—field trial of a pocket device. *Lepr. Rev.* **60** (1989) 317–326.

A handy thermal-sensibility testing device has been developed and field tested in different centers in Africa and India. The device performed satisfactorily under field conditions and made testing for thermal sensibility in the field practicable and easy. Examination of the results of testing 260 persons, most of them having a few lesions of early leprosy, showed that the expected increase in the rate of diagnosis of sensory impairment in the skin lesions, and so in the diagnosis of leprosy, would be about 15%–25% when thermal-sensibility testing using this device was added to the other sensibility tests routinely used in the field. Regular use of this device in the field will help to bring more leprosy patients under treatment than at present.—Authors' Summary

Suster, S., Cabello-Inchausti, B. and Robinson, M. J. Nongranulomatous involvement of the bone marrow in lepromatous leprosy. *Am. J. Clin. Pathol.* **92** (1989) 797–801.

Bone-marrow involvement in lepromatous leprosy has been characterized histologically by a proliferation of foamy histiocytes containing lepra bacilli, the so-called Virchow cells. The authors have studied three patients with biopsy-proven lepromatous leprosy in whom Fite stain, performed on histologic sections of bone-marrow aspirates, demonstrated numerous bacilli lying free in the interstitium in the absence of Virchow cells or focal collections of foamy macrophages. Two of the patients had a recent diagnosis of lepromatous leprosy by skin biopsy; the third patient had a 33-year history of lepromatous leprosy that had been treated. Bone-marrow aspirates were performed in all three patients for evaluation of anemia. The findings indicate that the bone marrow may act as a reservoir for viable organisms in the absence of a host response in treated and untreated patients with lepromatous leprosy. The persistence of viable organisms in the bone marrow in patients with lepromatous leprosy may account for the high rate of relapse and/or recrudescence of the disease following cessation of specific therapy. Bone-marrow examination with the Fite modification of the acid-fast stain is therefore indicated in such patients to evaluate bone-marrow involvement and the efficacy of treatment.—Authors' Abstract

Urmil, A. C., Dutta, P. K., Basappa, K. and Ganguly, S. S. A study of morbidity pattern among prostitutes attending a municipal clinic in Pune. *J. Indian Med. Assoc.* **87** (1989) 29–31.

The findings of the present study revealed that out of 200 prostitutes attending a clinic for various ailments, 81.50% were suffering from sexually transmitted diseases (STD), thus posing a potential risk of transmitting these diseases to their clients. Syphilis was found to be the commonest STD, afflicting 36.60% of the respondents, the next common being the chancroid (31.28%); 5.52%

of the respondents were found to be suffering from concomitant venereal infections. The other important communicable diseases with which some respondents were found to be afflicted, included—tinea infection (3 cases), scabies (2 cases), leprosy (2 cases), pulmonary tuberculosis (4 cases) and upper respiratory tract infection (3 cases). Thus, the prostitutes remain an undisputed potential source of infection, not only of STDs but also several other communicable diseases. Therefore, their continuous surveillance, early diagnosis, appropriate treatment and subsequent follow-up should be meticulously carried out. On the other hand the public, particularly sexually promiscuous individuals, must be imparted vigorous health education to avoid exposure to this source.—Authors' Abstract

Vetrom, L. and Pritze, S. Reliability of skin smear results: experiences with quality control of skin smears in different routine services in leprosy control programmes. *Lepr. Rev.* **60** (1989) 187–196.

The quality control of skin smears is an important tool in improving the diagnosis of leprosy. We evaluated the skin smears sent to us by 50 laboratory technicians of 29 projects in Asia, Africa and South America. The skin smears were judged according to taking, staining and reading. The correlation was altogether satisfactory. In reading, a low correlation was found in 11% (42 slides), and it was seen that the highest percentage of low correlations was found in the false-negative smears. The evaluation of cases with a low correlation leads to the conclusion that using the new WHO classification of 1988 will not reduce the number of incorrectly classified cases. From 42 slides showing a low correlation of their BI results, 7% led to a different classification (paucibacillary instead of multibacillary or vice versa) according to the WHO definition given in 1982, but 8% according to the 1988 WHO definition.—Authors' Summary

Immuno-Pathology

Bagshawe, A., Scott, G. C., Russell, D. A., Wigley, S. C., Merianos, A. and Berry, G. BCG vaccination in leprosy: final re-

sults of the trial in Karimui, Papua New Guinea, 1963–79. *Bull. WHO* **67** (1989) 389–399.

The efficacy of BCG vaccine in preventing the clinical manifestations of leprosy in a tuberculosis-free area of Papua New Guinea is reported. Between 1963 and 1966 a total of 5356 subjects, randomized to receive BCG or saline inoculations, were examined for leprosy before the vaccination and surveillance was continued until 1979. BCG afforded 48% protection against clinical leprosy, being most effective against borderline tuberculoid leprosy and in children vaccinated when under 15 years old. Protection was evident within 12 months in those vaccinated between the ages of 10 and 15 years but was delayed in other age groups. There was evidence for accelerated manifestations of tuberculoid leprosy in children vaccinated when under 5 years of age. Tuberculin sensitivity was more likely to be sustained following multiple BCG inoculations; vaccinees with sustained tuberculin sensitivity had the lowest incidence of leprosy, but protection was also evident in tuberculin-negative vaccinees. These results may have implications for ongoing trials of leprosy vaccine incorporating BCG.—Authors' Abstract

Bloom, B. R., Mehra, V., Melancon-Kaplan, J., Castes, M., Convit, J., Brennan, P. J., Rea, T. H. and Modlin, R. L. Mechanisms of immunological unresponsiveness in the spectra of leprosy and leishmaniasis. *Adv. Exp. Med. Biol.* **239** (1988) 263–278.

The recent advances in monoclonal antibodies, development of specific T-cell clones, and recombinant DNA technology provide tools for identifying and producing protective antigens. In the case of leprosy and leishmaniasis, it is important, perhaps best using T-cell clones, to identify those antigens required for induction of T-helper and T-killer cell responses that are likely to be important in protection. It is similarly important to identify those antigens or epitopes involved in inducing T-cell suppression. And in both cases, it is important to learn to what extent those determinants are a property of the antigens, or depend on genetic polymorphisms in T-cell receptors and MHC Class I and Class II antigens in the populations. Only with this knowledge will it be possible, with any degree of certainty, to develop recombinant vaccines as useful as the present ones, but hopefully sig-

nificantly less expensive. Because of the unique adjuvant properties of mycobacteria, it is our objective to develop a recombinant mycobacterial multivaccine vehicle, particularly BCG, capable of expressing recombinant genes for antigens from a variety of pathogens to which T-cell immunity is critical for protection.—(From the Article)

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

Phenolic glycolipid (PGL)-I, a *Mycobacterium leprae*-specific antigen currently used for serodiagnosis of preclinical leprosy, has thus far not been localized subcellularly in leprosy bacilli and their host cells. In this study, we developed an immunogold-labeling technique for qualitative identification of PGL-I sites in glutaraldehyde-osmium-fixed and araldite-embedded *M. leprae* and host macrophages in human skin biopsies. Such "hard-fixed," plastic-embedded skin and nerve biopsies from patients with varying cell-mediated immunity to leprosy are amply available worldwide. Our method involves etching of plastic sections with H₂O₂ incubation with swine serum to eliminate nonspecific labeling, and long (22 hr) incubation at room temperature with monoclonal antibodies to PGL-I. Gold labeling was seen predominantly on the cell walls of *M. leprae*, in vacuolar spaces of bacillated phagolysosomes, and occasionally on the cytoplasm and cell membrane of *M. leprae*. Host macrophage cytoplasm was labeled very infrequently. This technique allows studies on possibly persisting antigenic PGL-I in multibacillary leprosy patients during or after multidrug therapy. The method may also prove useful for subcellular localization of specific bacterial lipids in other mycobacterial diseases, including tuberculosis.—Authors' Abstract

Boddingius, J. and Dijkman, H. In situ locations of *Mycobacterium leprae*-specific antigen; immuno-electronoptical studies. *Acta Leprol. (Genève)* **7** Suppl. 1 (1989) 107–112.

Lipid or protein antigen sites in *Mycobacterium leprae* proper and in *M. leprae*-infected human or armadillo tissues were investigated by immunogold-electron microscopy. Simultaneous preservation of immunogenicity of antigens and conservation of ultrastructural details of *M. leprae* and host cells was aimed at by subjecting organisms and tissues, prior to immunolabelling, to differing fixation, embedding and ultramicrotomy techniques.

The *M. leprae*-specificity of monoclonal antibodies (MoAbs) utilized in the study was tested first. Hereto, ultracyrosections of *M. leprae*, *M. tuberculosis* and *M. nonchromogenicum* suspended in gelatin were employed. MoAb anti-phenolic glycolipid I (PGL-I) and MoAb anti-36 kDa were found to be specific for *M. leprae*. MoAb anti-65 kDa also labelled the cytoplasm of *M. tuberculosis*. After incubation with MoAb anti-lipid MAIS, employed as control MoAb, no gold labelling of leprosy bacilli or host cells was seen.

PGL-I immunogenicity was still present after "hard" fixation of *M. leprae* and host cells in glutaraldehyde-OsO₄ and after araldite embedding. This enabled the qualitative demonstration of PGL-I inside the cell wall and capsular area of *M. leprae* and in vacuoles of bacillated phagolysosomes of macrophages in araldite-embedded, human-skin biopsies and armadillo liver parenchymal cells. Sites of 65-kDa and, to a lesser extent, of 36-kDa protein antigens in *M. leprae* were demonstrable only in ultracyrosections of non-fixed organisms and not in araldite sections. Results are discussed and recommendations for future investigations on *M. leprae* antigen sites are presented.—Authors' Summary

Botasso, O. A. and Morini, J. C. [Immunopathology of leprosy.] *Medicina (B. Aires)* **49** (1989) 210–212. (in Spanish)

Studies including close contacts of leprosy patients were performed; an inverse relationship between degree and length of exposure to *Mycobacterium leprae* and the magnitude of the *in vivo*-specific immune response (Mitsuda reaction) was observed. On the other hand, this variable is greatly influenced by genetic determination. In the experimental adjuvant arthritis model, *M.*

leprae treatment was able to trigger a splenic suppressor-cell population with regulatory activity on the immune response. These results suggest that the erythema nodosum leprosum episode is related to an excessive humoral response and immunoregulatory alterations.—Authors' English Summary

Brett, S. J., Lamb, J. R., Cox, J. H., Rothbard, J. B., Mehlert, A. and Ivanyi, J. Differential pattern of T cell recognition of the 65-kDa mycobacterial antigen following immunization with the whole protein or peptides. *Eur. J. Immunol.* **19** (1989) 1303–1310.

The 65-kDa stress protein from *Mycobacterium bovis* (bacillus Calmette Guérin) elicited T-cell proliferation and antibody responses in seven B10 congenic mouse strains with different H-2 haplotypes. To analyze T-cell determinants on this antigen, seven peptides corresponding to six predicted T-cell epitopes, and one defined B-cell epitope were synthesized. Mice were either immunized with the whole antigen and the specificity of the response was ascertained in respect of the six peptides, or mice were immunized with seven of the peptides and tested for proliferative responses to the whole molecule. The results showed that three peptides carried epitopes to which mice responded following injection of the whole molecule and that immunization with two additional peptides could prime for *in vitro* stimulation with the native antigen. The latter result indicates the feasibility of generating T-cell responses to "cryptic" epitopes on proteins by immunizing with peptides. The peptide-specific T-cell responses were distinctly influenced by the H-2 haplotype of mouse strains. However, two peptides were recognized by several H-2 disparate mouse strains, and one peptide could be presented by both I-A and I-E molecules. Immunization with several peptides induced a crossreactive T-cell proliferative response to the homologous GroEL protein isolated from *Escherichia coli*. The amount of crossreactivity was influenced by the extent of sequence homology between mycobacterial and *E. coli* proteins and the major histocompatibility complex class II molecule used to present the peptide.—Authors' Abstract

Colloquium: new developments in immunoblotting and associated detection. Biochem. Soc. Trans. **16** (1988) 131–146.

This colloquium included an update and seven presentations, one of which discussed the application of immunoblotting to the diagnosis of *Mycobacterium leprae* infection, by antibody and antigen detection, and to analysis of the T-cell response to mycobacterial infection.—D. W. FitzSimons (Trop. Dis. Bull.)

Das, P. K., Rambukkana, A., Baas, J. G., Groothuis, D. G. and Halperin, M. Enzyme-linked immunosorbent assay for distinguishing serological responses of lepromatous and tuberculoid leprosy to the 29/33-kilodalton doublet and 64-kilodalton antigens of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **28** (1990) 379–382.

Immunoblot assays for the antibodies to *Mycobacterium tuberculosis* sonic extracts showed that all serum specimens of 40 lepromatous and of 28 tuberculoid leprosy patients reacted in a significant manner to 29/33-kilodalton (kDa) doublet and 64-kDa antigens, respectively. By using an enzyme-linked immunosorbent assay, we observed a significantly high immunoglobulin G antibody titer to the purified *M. tuberculosis* 29/33-kDa doublet and 64-kDa antigens in lepromatous and tuberculoid leprosy patients, respectively, as compared with normal subjects and tuberculosis patients. This enzyme-linked immunosorbent assay serology may be useful for distinguishing two polar types of leprosy and for diagnosing leprosy in general.—Authors' Abstract

Deng, Y., *et al.* [Examination of the subgroups of T cells in the peripheral blood in leprosy.] China Lepr. J. **5** (1989) 12–14. (in Chinese)

Three subgroups of T cells were classified with monoclonal antibodies in 40 active leprosy cases and 26 cured ones. The results indicated the cell numbers of all three subgroups both in active and cured patients were fewer than in the controls, with significant differences statistically. In the lepromatous cases with ENL, OKT4⁺ cells were increased, OKT8⁺ cells decreased and the

ratio of the OKT4⁺/OKT8⁺ was higher than in the cases without ENL, including cured ones and in the controls. In the patients with or without ENL including the cured ones, the three subgroups of T lymphocytes and the ratio of OKT4⁺/OKT8⁺ showed no difference. It is suggested that the immunological status of leprosy patients was not influenced by anti-leprosy treatment.—Authors' English Abstract

Denis, M., Forget, A., Pelletier, M., Gervais, F. and Skamene, E. Killing of *Mycobacterium smegmatis* by macrophages from genetically susceptible and resistant mice. J. Leukocyte Biol. **47** (1990) 25–30.

The bactericidal function of macrophages was investigated in congenic mice expressing the phenotype of susceptibility (B10.A, *Bcg*^s) or resistance (B10.A.*Bcg*^r) to mycobacterial infection. When splenic and peritoneal macrophages from these two mouse strains were infected *in vitro* with *Mycobacterium smegmatis*, the *Bcg*^r macrophages were shown to inactivate *M. smegmatis* more efficiently than their *Bcg*^s congenic counterparts. The mechanisms of this superior antimycobacterial activity were studied further. Addition of catalase did not abolish killing to a significant degree in either allelic type of macrophage, suggesting that hydrogen peroxide production was not involved in the killing activity controlled by the *Bcg* gene. Activation of *Bcg*^s macrophages by exposure to crude lymphokines rendered them equally as efficient as their *Bcg*^r counterparts in their capacity to destroy *M. smegmatis*. This finding suggests that both the genetically resistant and susceptible macrophages have the potential to kill *M. smegmatis in vitro*. This potential is expressed constitutively by the *Bcg*^r but not *Bcg*^s macrophages and can be induced, by lymphokine treatment, in the *Bcg*^s macrophages. In a final set of experiments, the macrophage killing of *M. smegmatis* was evaluated as a test system to type for the *Bcg* gene allelic type *in vitro*, using a set of AXB and BXA recombinant inbred strains of mice. Results obtained show that typing of AXB/BXA recombinant inbred strains for the trait of bactericidal activity versus *M. smegmatis in vitro* revealed a perfect match with the strain distribution pattern

of resistance/susceptibility to *M. bovis* BCG *in vivo*.—Authors' Abstract

Deo, M. G. ICRC anti-leprosy vaccine. Vaccine 7 (1989) 92–93.

A vaccine containing ICRC bacilli, which are leprosy-derived cultivable mycobacteria, induces lepromin conversion in LL patients and lepromin-negative persons. "Upgrading" of lesions and reversal reaction are observed in some vaccinated patients. The bacillus shows antigenic crossreactivity with *Mycobacterium leprae*. These observations provided the basis for launching a large-scale field trial of the vaccine in India in February 1987. The objective of the two-arm trial is to assess efficacy of the ICRC vaccine against BCG, which forms the control arm, in lowering the incidence of leprosy in healthy household contacts of leprosy patients. Recently, a very high molecular weight fraction, named PP-I, has been isolated from the sonicate of ICRC bacilli. PP-I, which is a glycolipoprotein, is a strong T-cell immunogen and shows antigenic crossreactivity with a similar fraction isolated from *M. leprae*. A "subunit" vaccine containing the PP-I of ICRC bacilli is currently undergoing phase I and II clinical trials in India.—Author's Abstract

Dudani, A. K. and Gupta, R. S. Immunological characterization of a human homolog of the 65-kilodalton mycobacterial antigen. Infect. Immun. 57 (1989) 2786–2793.

A human mitochondrial protein, designated P1 (63 kilodaltons [kDa]), shows extensive sequence homology (47% identical residues and an additional $\approx 20\%$ conserved changes) to the 65-kDa mycobacterial antigen. To understand the relationship of these proteins, the crossreactivity of several monoclonal antibodies directed against the 65-kDa *Mycobacterium leprae* antigen toward human, Chinese hamster, chicken, and bacterial cells has been examined. A number of antibodies (Y1-2, ML 30-A₂, and F47-9-1) were found to crossreact with a 63-kDa antigen in vertebrate cell extracts and stained mitochondria in immunofluorescence studies. Some of these antibodies also reacted with a P1- β -galactosidase fu-

sion protein in recombinant *Escherichia coli* cells, expressing part of the human P1 protein. These results provide strong evidence that P1 is the mammalian homolog of the 65-kDa antigen. The human P1 protein also shows significant similarity ($p < 0.001$) to a number of other bacterial and viral proteins including the pol polypeptide of human immunodeficiency viruses and the penicillin-binding protein of *Neisseria gonorrhoeae*. The observed similarity between human P1 protein and the major antigenic proteins of pathogenic organisms (e.g., 60- to 65-kDa mycobacterial antigen) suggests its possible involvement in autoimmune diseases (e.g., rheumatoid arthritis) by antigenic mimicry.—Authors' Abstract

Fernandez Bussy, R., Crisci, C., Firenza, G. and Bertoya, L. [Evaluation of circulating immune complexes in psoriasis, lichen planus, atopic eczema, leprosy, leukocytoclastic vasculitis, and pyoderma gangrenosum (review of the literature).] Rev. Argent. Dermatol. 70 (1989) 155–163. (in Spanish)

Several skin diseases associated with immune disorders may be related to the formation of circulating immune complex (CIC) and their skin deposition. Sera from 20 controls and 108 patients (including 23 psoriasis, 10 lichen planus, 30 atopic eczemas, 32 cases of leprosy, 10 vasculitis, and 3 pyoderma gangrenosum) were evaluated for the presence of IgG-containing CIC by the microconsumption complement test (MCT). Additionally, the presence of IgE-containing CIC by means of a polyethylene glycol precipitations and radioimmunoassay technique was evaluated in 10 patients with atopic eczema. It was found that 56.5% of psoriatic patients show moderate CIC concentrations, as well as 34% of leprosy patients, with increased levels when bacilli were detected in skin lesions, and in 90% of leukocytoclastic vasculitis. A close relationship between CIC levels and the clinical evolution of skin lesions was demonstrated in patients with pyoderma gangrenosum and lichen planus. IgG-CIC were detected in 33% and IgE-CIC in 30% of patients with atopic eczema, with simultaneous presence of both types of CIC in 2 out of 3 cases. The systematic research on CIC presence in some

selected skin diseases shows that IC take part, with different degrees of relevance, in the pathogenesis of them all.—Authors' English Summary

Fine, P. E. M., Gruer, P. J. K., Maine, N., Ponnighaus, J. M., Rees, R. J. W. and Stanford, J. L. Failure of *Mycobacterium leprae* soluble antigens to suppress delayed-type hypersensitivity reaction to tuberculin. *Clin. Exp. Immunol.* **77** (1989) 226–229.

In order to test a published claim that the inclusion of *Mycobacterium leprae* antigens with a tuberculin skin test reagent can suppress delayed-type hypersensitivity (DTH) to tuberculin in both paucibacillary and multibacillary leprosy cases, 109 leprosy cases and 104 nonleprosy controls were skin tested simultaneously with tuberculin with and without *M. leprae*-soluble antigens. Tests were randomized between arms and carried out double-blind. There was a clear tendency for larger DTH responses with the combined tuberculin plus *M. leprae* antigen than with tuberculin alone in paucibacillary leprosy cases and in nonleprosy controls. No evidence for *M. leprae* antigen-mediated suppression of DTH was observed in any group. It is unclear whether the difference between the results reported here, which were obtained in Malawi, and those in the published literature, which were obtained in India, is attributable to geographic differences in important biological variables or to differences in the experimental protocols. The need for methodological rigor in skin-test studies is stressed.—Authors' Summary

Goto, Y., Buschman, E. and Skamene, E. Regulation of host resistance to *Mycobacterium intracellulare* in vivo and in vitro by the *Bcg* gene. *Immunogenetics* **30** (1989) 218–221.

The purpose of this investigation was to determine whether the genetic control of resistance and susceptibility to *Mycobacterium intracellulare* is regulated by the *Bcg/Lsh* locus on mouse chromosome 1. We established that the *Bcg* gene controls early resistance to infection with *M. intracellulare* and that the *Bcg* gene is expressed *in vitro* in the form of an enhanced bacteriostatic

effect in *Bcg*⁺ peritoneal macrophages. These results extend previous reports showing superior anti-leishmania activity of macrophages isolated from congenic B10-*Lsh*-mice. However, since the *Bcg/Lsh/Ity* resistance mechanism is expressed in response to a variety of intracellular pathogens as well as activation signals, it is probable that the gene control of the macrophage activation process involves events subsequent to ligation of specific cellular receptors. The relationship of *Bcg* to *H-2* genes during the immune response to mycobacterial infection requires further investigation.—(From the Article)

Harris, D. P., Bäckström, B. T., Booth, R. J., Love, S. G., Harding, D. R. and Watson, J. D. The mapping of epitopes of the 18-kDa protein of *Mycobacterium leprae* recognized by murine T cells in a proliferation assay. *J. Immunol.* **143** (1989) 2006–2012.

The 18-kDa protein of *Mycobacterium leprae* was purified from recombinant plasmids pUL108 and pML-3 grown in *Saccharomyces cerevisiae* and *Escherichia coli*, respectively. Significant lymphoproliferative responses were observed when T cells from immunized mice were challenged in culture with purified 18-kDa protein. Synthetic peptides have been prepared that span most of the 148 amino acid residues that constitute the sequence of the 18-kDa protein and used to map epitopes recognized by T cells. When mice were immunized with 18-kDa protein and lymph node cells subsequently prepared and challenged in microculture proliferative assays by using synthetic peptides, only one region of the intact protein appeared stimulatory. This T-cell epitope was located between residues 116 and 121, adjacent to an epitope between residues 110 and 115 which we have previously shown to bind the L5 monoclonal antibody. Immunization of mice with peptides, and subsequent challenge of lymph node cells in assays by using the 18-kDa protein as antigen revealed that residues 111–125 were the most effective in priming responses. Furthermore, the ability of 18-kDa primed lymph node cells to recognize determinants on both *M. leprae* and *M. tuberculosis* indicates that in addition to pos-

sessing an *M. leprae*-specific B-cell determinant, the 18-kDa protein contains a crossreactive T cell epitope(s).—Authors' Abstract

Ivanyi, J., Sharp, K., Jackett, P. and Bothamley, G. Immunological study of the defined constituents of mycobacteria. Springer Semin. Immunopathol. **10** (1988) 279–300.

The identification of mycobacterial antigens and their fine specificity at the level of single epitopes has progressed mainly through the application of monoclonal antibodies, recombinant DNA, and T-cell cloning technologies. These advances have been essential, enabling a renewed analysis of the protective and pathogenic interactions of the infected host with mycobacterial pathogens. Distinct patterns of immunodominance in the immune repertoires, structural homologies with other exogenous and host antigenic constituents and the molecular targets for immunogenetic control have been indicated. The hypothesis, proposing a biological function within host cells for certain mycobacterial proteins (homologous with hsp), represents a new concept for the study of mycobacterial virulence. Clinically relevant aspects, such as immunodiagnosis, are being expressed in more precise molecular terms than before, while the preparation and adequate formulation of antigenic subunits with protective potency have not yet been achieved. However, the interim data based on a small fraction of the known constituents suggest that full biological evaluation of the current wealth of molecular information is yet some way from realization.—Authors' Concluding Comments

Kaplan, G., Mathur, N. K., Job, C. K., Nath, I. and Cohn, Z. A. Effect of multiple interferon- γ injections on the disposal of *Mycobacterium leprae*. Proc. Natl. Acad. Sci. U.S.A. **86** (1989) 8073–8077.

The effect of multiple intradermal injections (four to six) of 10 μ g of interferon γ on the number of *Mycobacterium leprae* in the skin of patients with polar lepromatous leprosy and borderline lepromatous leprosy was evaluated. To achieve a maximum zone of induration and cell emigration a prepa-

ratory dose of the lymphokine was required. A second group of three injections, given 3–4 days after the initial series, resulted in lesser degrees of induration and was more in keeping with a partial local hyporesponsive state. A marked emigration of T cells and monocytes into the dermis resulted from injections of interferon γ and persisted for > 21 days. A preponderance of CD4⁺ cells in the infiltrate was seen within a few days and CD4/CD8 ratios remained elevated for > 3 weeks. The bacillary load of injected sites evaluated 21 days after lymphokine administration was reduced in 14/17 patients by factors ranging from 5- to 1000-fold. This occurred predominantly within diffuse lesions and occurred rarely in nodular sites. Biopsy samples of injected sites taken 6 months later demonstrated progressive 10-fold reductions in bacilli and the continued presence of a granulomatous response.—Authors' Abstract

Kaufmann, S. H. E. Leprosy and tuberculosis vaccine design. Trop. Med. Parasitol. **40** (1989) 251–257.

Tuberculosis and leprosy are bacillary infectious diseases which cause severe global health problems with approximately 50 to 60 million people suffering from tuberculosis and 10 to 15 million from leprosy. In the developing countries the currently available vaccine, bacille Calmette-Guérin (BCG) was found to be less effective than originally thought. This disappointment, as well as recent achievements in biotechnology, has led several researchers to embark on novel avenues toward a rational vaccine design. This strategy stems from the idea that protective antigens exist which can be identified by immunological methods, expressed as recombinant gene products, and administered in a way that induces a protective T-cell response.—Author's Summary

Klatser, P. R., de Wit, M. Y. L., Fajardo, T. T., Cellona, R. V., Abalos, R. M., dela Cruz, E. C., Madarang, M. G., Hirsch, D. S. and Douglas, J. T. Evaluation of *Mycobacterium leprae* antigens in the monitoring of a dapsone-based chemotherapy of previously untreated lepromatous patient in Cebu, Philippines. Lepr. Rev. **60** (1989) 178–186.

Thirty-five previously untreated lepromatous patients receiving dapsone-based therapy were monitored throughout their 5-year period of treatment by serology and by pathology. Sequentially collected sera were used to evaluate the usefulness of four *Mycobacterium leprae* antigens as used in ELISA to monitor the progress of their therapy. ELISA results were compared with each other and with bacterial load over the treatment period and with duration of treatment. The ELISAs, based on the measurement of IgM antibody reactivity to the two neoglycoproteins (NDO and NTO) representing the phenolic glycolipid antigen of *M. leprae*, were found to be the most effective in monitoring treatment. A whole *M. leprae*-based ELISA was less efficient in monitoring treatment because it failed to measure antibodies in 8 out of 35 patients and because it provided consistently lower values than either NTO or NDO. The ELISA-inhibition test based on the detection of antibodies to a species-specific epitope on the 36-kDa antigen of *M. leprae* was less suitable because of persistent reactivity during therapy, consequently resulting in no significant correlation with ELISA reactivities to NTO or NDO.—Authors' Summary

Koga, T., Wand-Württenberger, A., De-Bruyn, J., Munk, M. E., Schoel, B. and Kaufmann, S. H. E. T cells against a bacterial heat shock protein recognize stressed macrophages. *Science* **245** (1989) 1112–1115.

Heat-shock proteins are evolutionarily highly conserved polypeptides that are produced under a variety of stress conditions to preserve cellular functions. A major antigen of tubercle bacilli of 65 kilodaltons is a heat-shock protein that has significant sequence similarity and crossreactivity with antigens of various other microbes. Monoclonal antibodies against this common bacterial heat-shock protein were used to identify a molecule of similar size in murine macrophages. Macrophages subjected to various stress stimuli including interferon- γ activation and viral infection were recognized by class I-restricted CD8 T cells raised against the bacterial heat-shock protein. These data suggest that heat-shock proteins are processed in stressed host cells and that

epitopes shared by heat-shock proteins of bacterial and host origin are presented in the context of class I molecules.—Authors' Abstract

Lecoeur, H. F., Lagrange, P. H., Trufflot-Pernot, C., Gheorghiu, M. and Grosset, J. Relapses after stopping chemotherapy for experimental tuberculosis in genetically resistant and susceptible strains of mice. *Clin. Exp. Immunol.* **76** (1989) 458–462.

Three strains of mice (Swiss albinos, C57BL/6, C3H/OuJ) were injected intravenously with 3.7×10^6 colony forming units (CFU) of *Mycobacterium tuberculosis* H37RV, sensitive and resistant to antibiotics (90% of bacilli sensitive, 9% resistant to streptomycin and 0.9% resistant to kanamycin). Two weeks later, chemotherapy was started 6 days a week for a 6-month period with isoniazid (INH) and rifampin (RMP). Twenty mice of each strain were killed at the end of the chemotherapy and the others were kept without antibiotics for a second 6-month follow-up period before being killed. The early multiplication of bacilli, during the first 2 weeks following infection and before chemotherapy, was similar in the three strains of mice. Chemotherapy had the same apparent efficacy in the three strains of mice, nearly all the mice being cured as assessed by a negative spleen culture on Löwenstein-Jensen medium at the end of chemotherapy. But after the 6-month follow-up period, the C3H strain presented a statistically significantly higher level of positive spleen culture ("relapse") than seen in the C57BL/6 strain, and an increased number of mycobacteria per relapsing mouse spleen. It has been estimated with the help of resistant and sensitive bacilli that the relapses were due in most of the cases to the regrowth of one or very few bacilli, giving a clone. It seems that the C3H strain of mice, known to carry the *Bcg-r* allele of the *Bcg* gene, might be less able to develop a specific acquired resistance capable of stopping the delayed development of a highly virulent strain of mycobacteria.—Authors' Summary

Li, M., et al. [Procoagulant activity of the lymphocytes in the peripheral blood of

leprosy patients.] China Lepr. J. 5 (1989) 15–17. (in Chinese)

The procoagulant activity of the lymphocyte in the peripheral blood was determined with the method of Gecze, *et al.* in 27 cases of leprosy (BB-LL) and 37 healthy controls. The results show that those with the value of the activity over zero seconds were 40.7% and 51.4% in the two groups, respectively, while PHA was used as a stimulus ($p > 0.5$). The figures were 37% and 62.2% ($p < 0.05$) when taking soluble protein of *Mycobacterium leprae* as the stimulus. The authors think that the result indicates a functional defect of specific cell-mediated immunity in leprosy. The method is simple, rapid and economical in operation and could be used to examine the function of cell-mediated immunity as an additional test.—Authors' English Abstract

Liu, Z., *et al.* [Pathology of the peripheral nerve lesions in infected armadillo with *M. leprae*—a light- and electron-microscopic study.] China Lepr. J. 5 (1989) 69–71. (in Chinese)

The lesions of peripheral nerves in armadillos infected with *Mycobacterium leprae* have been studied by light- and electron microscope. We found some lesions in axons of nerve fibers which have not been reported in the literature, including invasion of axons by *M. leprae*, various sizes of vesicle formations, rarefaction or condensation of neurofilaments, as well as the presence of myelin figures inside the axons. All these provide further understanding of the development and progression of nerve lesions in the armadillo and will be helpful in the study of the pathogenesis and development of lepromatous nerve lesions. Evidence for the spread of leprosy lesions to the peripheral nerves is shown to be not only by the hematogenous route, but may also be by way of the lymphatics involved in leprosy lesions.—Authors' English Abstract

Lucas, S. B. and Ridley, D. S. The use of histopathology in leprosy diagnosis and research. (Editorial) Lepr. Rev. 60 (1989) 257–262.

The difficulty and lack of agreement in diagnosing early (paucibacillary) leprosy by

histopathology is frustrating. There is no reason why the essentially subjective nature of histological observation and diagnosis should change in the future. Whether the newer techniques of detecting *Mycobacterium leprae* DNA or antigens will facilitate early diagnosis is not yet known; if they can help in a research center, will they be useful in more peripheral laboratories? The study of cell types and mediators operating in leprosy lesions tells us about pathogenetic immune mechanisms, and may have therapeutic spinoffs. The global provision of histopathology is woefully inadequate. For leprosy, its present vertical arrangement is unlikely to change in the near future, and many endemic countries will continue to be dependent on the pathological expertise of developed countries.—Authors' Conclusions

Meeker, H. C., Williams, D. L., Anderson, D. C., Gillis, T. P., Schuller-Levis, G. and Levis, W. R. Analysis of human antibody epitopes on the 65-kilodalton protein of *Mycobacterium leprae* by using synthetic peptides. Infect. Immun. 57 (1989) 3689–3694.

In order to study antibody reactivity to the *Mycobacterium leprae* 65-kilodalton (kDa) antigen, peptides representing overlapping sequences of the 65-kDa protein were synthesized, and a recombinant protein expression system for r65-kDa was constructed. Mouse monoclonal antibodies and leprosy patient seroreactivity to peptides and r65-kDa were tested by an enzyme-linked immunosorbent assay. All seven of the monoclonal antibodies used in this study reacted with their previously defined epitopes when tested against peptides. All monoclonal antibodies also reacted with r65-kDa. Leprosy patient seroreactivity to peptides and r65-kDa was seen in about one third of active multibacillary cases. Specimens from patients positive for antibodies to peptides were seen to recognize different epitopes than did mouse monoclonal antibodies used in this study. It is concluded that substantial differences exist between mouse monoclonal antibodies and human leprosy patient reactivity to the 65-kDa antigen and that human seroreactivity to the

65-kDa antigen is indicative of a highly elevated bacillary load.—Authors' Abstract

Moudgil, K. D., Gupta, S. K., Narayanan, P. R., Srivastava, L. M., Mishra, R. S. and Talwar, G. P. Antibody response to phenolic glycolipid I and *Mycobacterium w* antigens and its relation to bacterial load in *M. leprae*-infected mice and leprosy patients. *Clin. Exp. Immunol.* **78** (1989) 214–218.

Twenty-six inbred BALB/cBy mice were infected with live *Mycobacterium leprae* by injecting 6×10^3 bacilli in the hind foot pad. Bleeds were collected at monthly intervals. After 6 months, acid-fast bacilli (AFB) were harvested monthly from the foot pads of mice. The sera were analyzed in enzyme immunoassay for antibodies against phenolic glycolipid I (PGL-I) of *M. leprae* and antigens of "*Mycobacterium w*" (*M. w*); 21 out of 26 (80.7%) mice demonstrated the presence of antibodies against PGL-I and *M. w*. Anti-*M. w* antibodies appeared slightly earlier than did anti-PGL-I antibodies. The titer of anti-*M. w* antibodies was higher than that of anti-PGL-I antibodies. The mice giving a positive antibody response had more than 7×10^5 AFB/foot pad. The coefficient of correlation (*r*) between the number of AFB and antibody titers at the time of harvest was 0.566 for PGL-I and 0.628 for *M. w*. The value of *r* for bacterial index and antibody titers in 188 leprosy patients was 0.510 for PGL-I and 0.418 for *M. w*; these values were statistically significant ($p < 0.001$). The decrease in bacterial index and antibody titers in treated lepromatous leprosy patients correlated with increase in the duration of chemotherapy. The measurement of anti-PGL-I antibodies of IgM class may serve as an adjunct to skin biopsy and skin-slit smear for serial monitoring of the bacterial load in the course of chemotherapy in leprosy control programs.—Authors' Summary

Pessolani, M. C. V., Rumjanek, F. D., de Melo Marques, M. A., de Melo, F. S. F. and Sarno, E. N. Serological response of patients with leprosy to a 28- to 30-kilodalton protein doublet from early cultures of *Mycobacterium bovis* BCG. *J. Clin. Microbiol.* **27** (1989) 2184–2189.

Analysis by Western immunoblotting of *Mycobacterium bovis* BCG short-term culture filtrates with a pool of serum samples from lepromatous leprosy patients revealed an immunodominant protein doublet with apparent molecular masses of 28 and 30 kilodaltons (kDa). The humoral response to these antigens was also investigated by using individual serum samples from patients representative of the whole spectrum of leprosy and from tuberculosis patients, as well as from contacts of leprosy patients and control groups. The protein doublet was recognized by 92% of the sera from patients with lepromatous leprosy (51 of 56); whereas essentially negative results were obtained with sera from the other groups. Similar immunodominant bands were also detected by Western blotting analysis of sonic extracts of seven other slow- and fast-growing mycobacterial species, suggesting a broad distribution of these antigens within the genus. Analysis of the purified doublet by Western blotting after two-dimensional gel electrophoresis fractionation showed that the 28- and 30-kDa doublet consisted of at least five different components with pIs from 5.2 to 5.7 and molecular masses from 28 to 31 kDa. These results indicate that the protein doublet could be used as a potential marker in the diagnosis of lepromatous leprosy.—Authors' Abstract

Poulter, L. W. and Condez, A. The effect of intracellular parasitism on cell phenotype. *Clin. Exp. Immunol.* **74** (1988) 344–348.

Human peritoneal macrophages were harvested from "long dwell" peritoneal dialysis bags. Adherent cells were harvested after 2-hr culture on plastic and these were then exposed to *Mycobacterium leprae*-murium (MLM) or latex beads (LB) for further culture periods of 2 hr and 24 hr. Immunophenotyping of the macrophages was performed before and after culture both with and without the addition of MLM or LB. Monoclonal antibodies RFD1, RFD7 and RFD9 were used, which in control tissues recognize dendritic cells, mature macrophages and epitheloid cells, respectively. Monoclonal antibody RFDR1 (HLA-DR) was also used. Results revealed that the addition of MLM or LB for 2 hr did not sig-

nificantly alter the original proportions of RFD1+ cells and RFD7+ cells. A proportion of both RFD1+ and RFD7+ cells were found to phagocytose both MLM and LB, although RFD7+ cells seemed slightly more efficient. After 24-hr culture with MLM, reduced numbers of cells expressed the RFD1+ marker while increased numbers of cells expressing RFD7+ antigen were present. A concurrent small rise in the proportion of RFD7+ cells parasitized implied that this increase was due to RFD1+ cells becoming RFD7+ cells. Culture for 24 hr with MLM significantly reduced the percentage of total cells expressing HLA-DR. Conversely, 24 hr culture with MLM was shown to increase the proportion of cells expressing the epithelioid cell marker RFD9. These results suggest that intracellular parasitism may affect the expression of surface antigens on cells and by implication thus affect cell function.—Authors' Summary

Poulton, T. A., Gallagher, A. and Beck, J. S. Suppression of the increase in free cytosolic calcium during the inhibition of T-cell activation by an autoantibody present in the serum of leprosy patients. *Immunology* **68** (1989) 353–358.

A serum factor, believed to be an IgG autoantibody, in certain patients with lepromatous leprosy inhibits the proliferation of mitogen-stimulated lymphocytes. To investigate which stage of the cell cycle was inhibited, we examined the effect of these sera on the kinetics of lymphocyte activation induced by several mitogenic agents: phytohemagglutinin (PHA), the calcium ionophore A23187, the phorbol ester phorbol myristate acetate (PMA), and purified protein derivative of BCG (PPD). Seven out of 54 sera tested were found to inhibit PHA-stimulated proliferation. Inhibitory sera and to a lesser extent serum IgG from leprosy patients were capable of suppressing the increase in free cytosolic calcium normally observed immediately after PHA stimulation. Subsequent stages of the cell cycle, increase in cell size, the expression of the IL-2 receptor, and increase in DNA were also suppressed. The inhibitory sera was not toxic and, if addition of the sera was delayed, would not inhibit lymphocytes that had already entered the cell cycle. Using mitogen-

ic agents which act intracellularly, the normal early increase in cell size with A23187- and PMA-stimulated lymphocytes was not affected by inhibitory leprosy sera or serum IgG, but all subsequent steps in the cell cycle were suppressed, although the inhibition of proliferation in PMA-stimulated cultures was incomplete. The mechanism of action of the inhibitory sera and derived IgG, although acting through a cell surface antigen, appears to interfere with a fundamental process in activation since the effect was seen with all of the diverse stimuli examined in this study.—Authors' Summary

Rabello, F. E. and Azulay, R. D. [Comparative ecology of tuberculosis and hanseniasis.] *Med. Cutan. Ibero Lat. Am.* **17** (1989) 145–157. (in Portuguese)

The authors discuss the several aspects of the co-sensibilization between the infections produced by *Mycobacterium tuberculosis* and *M. hanseniae*. Attention is called to Rabello's pioneer works written in 1935 and the subsequent works written by several Argentinian and Brazilian authors. Special emphasis is given to the experimental works in animals by Azulay. The authors discuss the aspects of competitiveness, protection and the crossed granulomatous responses to the infections.—Authors' English Summary

Rawlinson, W. D. and Basten, A. Cross-reactivity of *Mycobacterium leprae* and *M. tuberculosis* and the implications for assessment of *in vitro* T cell function in leprosy patients. *Trans. R. Soc. Trop. Med. Hyg.* **83** (1989) 557–559.

The crossreactivity *in vitro* between *Mycobacterium leprae* and *M. tuberculosis* was studied in 41 Aboriginal Australians with leprosy, 78 uninfected contacts of leprosy patients, and 38 control individuals. A vigorous T-cell response to epitopes crossreactive between these two mycobacteria was found for healthy uninfected contacts or noncontacts (controls) of leprosy patients, but not for the patients themselves. The data suggest that a vaccine based on antigen shared between *M. leprae* and other mycobacteria is unlikely to be useful in preventing leprosy. Further studies of responses *in vitro* to purified T-cell-reactive

antigens would be useful in designing newer vaccines for more widespread field studies of leprosy prevention.—Authors' Abstract

Richard, L., Forget, A. and Turcotte, R. Partial characterization of suppressor factors in spleen cell culture supernatants of *Mycobacterium lepraemurium*-infected mice. *Adv. Exp. Med. Biol.* **239** (1988) 279–285.

Suppressor factors (SF) were released into the culture supernatant when spleen cells from *Mycobacterium lepraemurium*-infected C57BL/6 mice were incubated at 37°C in the absence of any inducing agents. Some of the SF appeared to be specific in that they inhibited the blastogenic response to mycobacterial antigens by opposition to the others which inhibited the blastogenic responses to PHA, ConA and LPS. All SF seemed to be produced in a cyclic manner. In mice infected 9 weeks earlier, the release of "specific" SF occurred early (4–8 hr) during the incubation period; whereas the non-specific SF were released later on (12–32 hr). Adoptive transfers of SF-containing culture supernatants depressed the expression of delayed-type hypersensitivity to *M. lepraemurium* antigens but not its induction.—Authors' Summary

Rook, G. A. W., Al Attiyah, R. and Foley, N. The role of cytokines in the immunopathology of tuberculosis and the regulation of agalactosyl IgG. *Lymphokine Res.* **8** (1989) 323–328.

Tuberculosis is characterized by necrosis in the lesions and in skin-test sites, and by fever and weight loss. In contrast, other diseases with chronic T-cell-mediated responses, such as uncomplicated leprosy and sarcoidosis, have nonnecrotizing lesions with little systemic upset. Crude sonicates of *Mycobacterium tuberculosis* and *M. leprae* prepare skin sites for TNF-mediated damage via a pathway which unexpectedly appears to involve CD8+ T cells, and both mycobacteria contain potent triggers of TNF release (lipoarabinomannan and peptidoglycan derivatives). These observations can partially explain the pathology of tuberculosis, but fail to explain why similar events do not normally occur in leprosy. It now

seems likely that the answer lies in the existence of novel regulatory pathways. A recently recognized correlate (or consequence) of diseases characterized by T-cell-dependent, tissue-damaging pathology and cytokine release is an increase in the level of agalactosyl IgG. This behaves like a T-cell-dependent, acute-phase reactant and is raised in tuberculosis, rheumatoid arthritis, and Crohn's disease but not in sarcoidosis or uncomplicated leprosy. Thus it may act as a marker for a type of pathology of very broad significance, although its functional role remains obscure.—Authors' Abstract

Sagaro Delgado, B., Diaz de la Rocha Quevedo, A., Collazo Caballero, S., Rodriguez Garcia, M. A., Perez Acuna, H. and Martinez Granja, R. [Importance of histopathology in the immunologic spectrum of leprosy. Practical application.] *Rev. Cubana Med. Trop.* **39** (1987) 91–104. (in Spanish)

The general characteristics of histopathologic groups of the immunological spectrum of leprosy, according to Ridley, are presented. The histological picture is pointed out as the best indicator of the immunity state of the patient. Importance of correlation between histopathology and bacteriology and its value for the definition of the spectrum groups, as well as the histopathological index to determine amount of bacilli in a lesion, are also pointed out. Possibility of applying the Ridley classification in the medical practice by the histomorphologic identification of the groups constituting it, is emphasized. Criteria on immunologic characterization of cell populations confirming the forementioned groups are expressed. Biopsies of patients studied at the "Hermanos Ameijeiras" Hospital, Department of Dermatology, evaluated by these criteria are presented.—Authors' English Summary

Santiago, M. B., Cossermelli, W., Tuma, M. F., Pinto, M. N. and Oliveira, R. M. Anticardiolipin antibodies in patients with infectious diseases. *Clin. Rheumatol.* **8** (1989) 23–28.

IgG or IgM anticardiolipin antibodies were present in the sera of 67% of 33 pa-

tients with Hansen's disease, in 53% of 30 patients with tuberculosis, and in 50% of 16 patients with endocarditis. Despite the high frequency of these antibodies, no patient had a history of thrombosis or abortion. Anti-denatured DNA antibodies were tested in patients with tuberculosis and patients with Hansen's disease. Only in the latter group did we observe a statistically significant association between anticardiolipin and anti-denatured DNA antibodies. Anticardiolipin binding activity, however, could not be inhibited by preincubation of sera with a variable concentration of denatured DNA. These data suggest that: a) anticardiolipin antibodies in infectious diseases do not necessarily participate in the pathogenesis of thrombotic or obstetric complications; b) anti-denatured DNA and anticardiolipin antibodies in the population studied do not have a crossreaction.—Authors' Summary

Schwerer, B., Chujor, C. S. N., Bernheimer, H., Radl, J., Haaijman, J. J., Meeker, H. C., Sersen, G. and Levis, W. R. IgA antibodies against phenolic glycolipid I from *Mycobacterium leprae* in serum of leprosy patients and contacts: subclass distribution and relation to disease activity. Clin. Immunol. Immunopathol. **53** (1989) 202–211.

The anti-PGL-I IgA response against phenolic glycolipid I (PGL-I), a specific surface antigen of *Mycobacterium leprae*, was demonstrated to be essentially of the IgA₁ subclass in sera from leprosy patients and contacts. Anti-PGL-I IgA₁ mean levels were found to increase significantly from the tuberculoid toward the lepromatous pole of the leprosy disease spectrum, thus resembling the predominating anti-PGL-I IgM response. Furthermore, anti-PGL-I IgA₁ values were shown to increase significantly with increasing bacillary load, measured as bacillary index (BI) from skin biopsies. However, a number of BI-negative leprosy patients recorded elevated anti-PGL-I IgA₁ levels, possibly reflecting a persistence of disease activity. Three of 28 household or family contacts of leprosy patients were detected seropositive for anti-PGL-I IgA₁. Thus, our results suggest that anti-PGL-I IgA₁ may be considered as an additional parameter for the early detection of infection with *M. leprae*.—Authors' Abstract

Segal-Eiras, A., Croce, M. V., Ramos, G. S. and Corsico, B. [Isolation and characterization of immune complexes associated with malignant tumors and leprosy.] Medicina (B. Aires) **49** (1989) 125–130. (in Spanish)

We are dedicated to the study of circulating immune complexes (CIC) associated with different diseases: malignant tumors, leprosy and rheumatoid arthritis. Immune complexes were evaluated by various methods: ¹²⁵I-Clq binding assay, ¹²⁵I-IgG binding test, ¹²⁵I-bovine conglutinating binding assay and polyethylene glycol precipitation test (3.5% and 2.5%). Techniques for the isolation and splitting of CIC in their components were performed in sera from patients with tumors and with leprosy. These methods consisted in the combination of CIC with protein A followed by elution with different buffers. CIC splitting techniques were first applied on immune complexes formed *in vitro* (BSA-aBSA, OVA-aOVA). The analysis of CIC fractions was done by SDS-PAGE, immunoelectrophoresis, and immunoblotting techniques. Results were as follows: CIC levels correlated with active stages of disease, decreasing during remission so that CIC detection can be useful to evaluate response to treatment. The isolation and splitting of immune complexes into their components resulted in the obtention of immunologically active fractions, especially in sera from patients with gastrointestinal and breast cancer and with leprosy.—Authors' English Summary

Stanford, J. L. Immunotherapy for leprosy and tuberculosis. Prog. Drug Research **33** (1989) 415–448. (71 refs.)

Although one of the first scientifically designed systems for treating both leprosy and tuberculosis, immunotherapy has still to prove itself. The position is very different today from that facing its originators in the 1890s. No longer does immunotherapy have to be a complete treatment, no longer does it have to be given repeatedly, and no longer does it have to be given in the presence of large bacterial and antigenic loads. As an adjunct to effective chemotherapy to shorten the course of treatment and to harness immune mechanisms to eradicate persisting bacilli that are metabolizing too slowly for

drugs to be effective is its potential for the future. It is difficult to see an alternative to immunotherapy in this role, and it is difficult to see what part the pure proteins of the so-called second-generation vaccines are going to play in the fight against the diseases that remains so far from over.—Author's Conclusion

Valdes-Portela, A. and de la Cruz, F. [Cell-mediated immunity in patients with leprosy treated with rifampin.] *Rev. Cubana Med. Trop.* **39** (1987) 23–32. (in Spanish)

Different parameters of cell-mediated immunity were studied in leprosy patients classified as TT/BT and LL, according to Ridley-Jopling scale, treated with rifampin. Tests, such as lymphoproliferation *in vitro* in response to phytohemagglutinin and lepromin, E, EA and EAC rosettes, delayed hypersensitivity and inhibition of leukocyte migration, were used. In the blastic transformation, it was observed, after treatment, an increased sensitivity in the response to phytohemagglutinin, since to obtain the maximal response only 12.5 µg/ml was needed, instead of 509 µg/ml, although the level reached in such maximal response remained significantly depressed in relation to controls. All the time, E rosettes were observed to be depressed in LL, while in the cases of TT/BT, values were normal in the three forms of rosettes. In delayed hypersensitivity a varying response and high values in the inhibition of leukocyte migration were observed in the cases classified as LL.—Authors' English Summary

Wong, L., Salgame, P., Torigian, V. K., Fu, T. H., Rea, T. H. and Modlin, R. L. CD2 expression and function in lepromatous leprosy. *Infect. Immun.* **57** (1989) 2815–2819.

Leprosy is a spectral disease in which clinical presentation is thought to be related to the host immune response. Previous investigations have suggested that selective unresponsiveness to *Mycobacterium leprae* in patients with lepromatous leprosy is due to the presence of *M. leprae*-specific T-suppressor cells. However, it has recently been suggested that CD2 modulation was the mechanism for the observed impaired immune response in lepromatous patients.

Therefore, we studied the expression of CD2 and CD3 on lymphocytes in lepromatous skin lesions and peripheral blood mononuclear cells (PBMC). Using immunohistochemical techniques, we found that virtually all of the CD3+ cells in leprosy skin lesions expressed CD2. In addition, indirect immunofluorescence flow cytometry demonstrated that most CD3+ cells in the peripheral blood possessed the CD2 marker, suggesting that CD2 expression of T lymphocytes is normal. T-cell activation using paired anti-T11₂ and anti-T11₃ or anti-CD3 monoclonal antibodies demonstrated similar ³H-thymidine incorporation and gamma-interferon production in the PBMC of lepromatous patients in comparison with the PBMC of their contacts and tuberculoid patients. However, lepromatous PBMC did not proliferate or produce gamma interferon in response to *M. leprae*. Our data suggest not only that CD2 expression is normal on T lymphocytes in lepromatous leprosy skin lesions but also that CD2 expression in peripheral blood lymphocytes is functional in T-cell activation. Defective CD2 modulation does not appear to be the mechanism for specific unresponsiveness in lepromatous leprosy.—Authors' Abstract.

Wu, Q., et al. [FLA-ABS T/PGL-ELISA and their uses in immuno-epidemiological studies for leprosy.] *China Lepr. J.* **5** (1989) 72–76. (in Chinese)

A comparison study between FLA-ABS, test and PGL-ELISA for their reliability and practicality has been conducted in 284 cases of leprosy, 20 of tuberculosis, 172 healthy persons as controls from an area without leprosy patients, 425 household contacts (HC) and 2573 persons of random population (PF). The results indicated that the two tests are highly sensitive and specific for detecting antibodies against *Mycobacterium leprae*.

Their correct indices (CI) and expected values of positivity and negativity are all higher than 90%. In addition, several agreements between the two tests have been found in leprosy immuno-epidemiological studies as well: a) The positive rates increased gradually from TT to LL of leprosy in FLA-ABS test and PGL-ELISA, and the PGL-ELISA's positive rate in HC of multibacillary patients is higher than that in HC of pauci-

bacillary ones. b) The positive rates of FLA-ABS test and PGL-ELISA are identical either in HC or in RP. c) For RP in different districts, the positive rates of the tests are similar and concordant with the general trend of the prevalence in the locality.

On the basis of above-mentioned results, the FLA-ABS test and the PGL-ELISA might be regarded as useful tools in the diagnosis of leprosy, detection of subclinical infection with *M. leprae* and relevant immuno-epidemiological studies. However, because it is simpler, rapid, cheaper and easier to its use, the PGL-ELISA is more practical than FLA-ABS test. On the other hand, it is emphasized that the methodology of dried blood from the earlobe is very important in achieving leprosy field immuno-epidemiological studies on a large scale. The authors put forward two preliminary concepts of "subclinical infection zone" and "diagnostic line" for the test. — Authors' English Abstract

Wu, Q.-X., Li, X.-Y., Shu, H.-W., Liu, Q., Xue, Z.-Y., Ma, Z.-X., Wei, W.-H., Ye, G.-Y. and Zhu, X.-S. Evaluation of fluorescent leprosy antibody absorption test versus enzyme-linked immunosorbent assay with phenolic glycolipid 1 and their use in immuno-epidemiological studies on leprosy. *Proc. CAMS and PUMC* 4 (1989) 106–111.

We systematically conducted comparative studies on the validity, reliability and practicality of FLA-ABS.T PGL-I-ELISA in large samples. Namely, 284 leprosy patients, 20 tuberculosis patients, 172 normal controls (from nonendemic area of leprosy), 425 leprosy household contacts (HC), and 2573 random samples from the general population (RS) were involved. The results indicated that FLA-ABS.T PGL-I-ELISA are highly sensitive and specific for detecting antibodies against *Mycobacterium leprae*. Their Youden's indexes (YI) are greater than 90%, and the positive predicative and negative values are 90%. The test results agreed with immuno-epidemiological studies: 1) The positive rates using FLA-ABS.T PGL-I-ELISA increased gradually from TT to LL leprosy patients (in HC, the positive rates of PGL-I-ELISA were much higher in contacts of multibacillary patients than in con-

tacts of paucibacillary patients); 2) The positive rates detected by FLA-ABS.T were identical to those of PGL-I-ELISA both in HC and in RS; 3) Among RS, the positive rates detected by FLA-ABS.T PGL-I-ELISA were similar in each district and were in concordance with the general prevalence rates. Thus, both FLA-ABS.T and PGL-I-ELISA are useful tests in diagnosing leprosy and detecting subclinical infection with *M. leprae*. However, because the PGL-I-ELISA is simple, it will be more practical than FLA-ABS.T in the future. The authors emphasize that the methodology of obtaining dried blood from earlobes is important for the immuno-epidemiological study of leprosy on a large scale. — Authors' Abstract

Yong, A. J., Grange, J. M., Tee, R. D., Beck, J. S., Bothamley, G. H., Kemeny, D. M. and Kardjito, T. Total and antimycobacterial IgE levels in serum from patients with tuberculosis and leprosy. *Tubercle* 70 (1989) 273–279.

A radioallergosorbent assay (RAST) was developed and used to determine the levels of IgE antibodies to soluble antigens of *Mycobacterium tuberculosis* (BCG vaccine strain) in sera from patients with tuberculosis and leprosy and in healthy control subjects. Total IgE levels in the same sera were quantitated with a commercial radioimmunoassay kit. Patients with tuberculosis and leprosy had higher total and specific IgE levels than the control groups, but the overlap of levels in patients and controls was too great to render the difference diagnostically useful. Specific IgE levels were elevated in both tuberculosis and leprosy patients, suggesting that this antibody response is toward the shared mycobacterial antigens. No differences in total or specific IgE levels were found between healthy hospital workers occupationally exposed to patients with tuberculosis and factory workers who are not exposed. — Authors' Summary

Zhu, W., *et al.* [Observation of the free zone in the skin section of leprosy patients with light- and electronmicroscopy.] *China Lepr. J.* 5 (1989) 10–12. (in Chinese)

The free zone in the skin sections of leprosy were histopathologically examined. The

results showed that the mean width is $48.11 \pm 20.96 \mu\text{m}$ in 30 cases of LL and $48.22 \pm 18.35 \mu\text{m}$ in 20 of BT ($p > 0.05$). The free zone manifests as atrophy of the epidermis, decrease of the vessels, no exudation of red cells, and a clear border of infiltration in

LL, but as generally normal epidermis, increase of the vessels, often exudation of red cells and unclear border of infiltrations in contact with the epidermis somewhere in BT.—Authors' English Abstract

Microbiology

Clark-Curtiss, J. E. and Walsh, G. P. Conservation of genomic sequences among isolates of *Mycobacterium leprae*. J. Bacteriol. **171** (1989) 4844–4851.

Restriction fragment length polymorphism analysis has been used to assess relatedness among the genomes of four isolates of *Mycobacterium leprae*, the causative agent of leprosy. The *M. leprae* isolates were from human patients from India, a mangabey monkey from west Africa, and an armadillo from Louisiana. A total of 16 probes were used; these were insert fragments of *M. leprae* DNA from plasmid recombinant libraries, 5 of which had genes with identifiable functions and 11 of which were randomly chosen recombinant molecules. In spite of the widely diverse origins of the isolates, restriction fragment length polymorphism analysis demonstrated that less than 0.3% of the nucleotides differ among the genomes.—Authors' Abstract

Hance, A. J., Grandchamp, B., Lévy-Frèbault, V., Lecossier, D., Rauzier, J., Bocart, D. and Gicquel, B. Detection and identification of mycobacteria by amplification of mycobacterial DNA. Mol. Microbiol. **3** (1989) 843–849.

A 383bp segment of the gene coding for the 65-kDa mycobacterial antigens from *Mycobacterium tuberculosis*, *M. bovis*, *M. avium*, *M. paratuberculosis*, and *M. fortuitum* was amplified using *Taq* polymerase and synthetic oligonucleotide primers and the amplified DNAs from four of these species were compared by nucleotide sequencing. Although the gene segments from these species showed considerable similarity, oligonucleotide probes which could distinguish *M. tuberculosis*/*M. bovis*, *M. avi-*

um/*M. paratuberculosis* and *M. fortuitum* could be identified. Samples containing 10^6 human cells and serial dilutions of a suspension of intact mycobacteria were prepared, DNA was extracted, the segment of the mycobacterial DNA sequence amplified, and the amplified DNA hybridized with oligonucleotide probes. In two independent experiments, this procedure permitted the detection and identification of less than 100 mycobacteria in the original sample. These results suggest that this approach may prove useful in the early diagnosis of mycobacterial infection.—Authors' Summary

Hartskeerl, R. A., de Wit, M. Y. L. and Klatser, P. R. Polymerase chain reaction for the detection of *Mycobacterium leprae*. J. Gen. Microbiol. **135** (1989) 2357–2364.

A polymerase chain reaction (PCR) using heat-stable *Taq* polymerase is described for the specific detection of *Mycobacterium leprae*, the causative agent of leprosy. A set of primers was selected on the basis of the nucleotide sequence of a gene encoding the 36-kDa antigen of *M. leprae*. With this set of primers in the PCR, *M. leprae* could be detected specifically with a detection limit approximating one bacterium. This PCR appears to meet the criteria of specificity and sensitivity required for a useful tool in epidemiology and eventually for the control of leprosy.—Authors' Abstract

Kikuchi, S., Takeuchi, T., Yasui, M., Kysaka, T. and Kolattukudy, P. E. A very long-chain fatty acid elongation system in *Mycobacterium avium* and a possible mode of action of isoniazid on the system. Agric. Biol. Chem. **53** (1989) 1689–1698.

A malonyl-CoA-dependent, acyl carrier protein (ACP)-non-requiring fatty acid elongation system was isolated from *Mycobacterium avium*. Chromatographical fractionation and reconstitution studies indicated that the system is composed of separable protein components, like a fatty acid elongation system from *M. smegmatis* reported previously. In comparison of the two systems, however, some distinct features were observed in pyridine-nucleotide-coenzyme requirements and in sensitivities to isoniazid (*N*-amino-3-pyridine carboxylic acid amide); the system from *M. avium* used NADPH as a sole hydrogen donor and was inhibited by the agent, *in vitro*, at the concentration of 2 mM, while the agent has been proved to have no inhibitory effect on the system from *M. smegmatis* which required both NADH and NADPH. Using the fractionated component enzymes of the system from *M. avium*, similar concentrations of isoniazid were found to be distinctly inhibitory against 3-oxoacyl-CoA reductase and more slightly against enoyl-CoA reductase with *K_i* values of 352.8 μ M and 5.5 mM, respectively.—Authors' Abstract

Larsson, L., Jimenez, J., Sonesson, A. and Portaels, F. Two-dimensional gas chromatography with electron capture detection for the sensitive determination of specific mycobacterial lipid constituents. *J. Clin. Microbiol.* **27** (1989) 2230–2233.

The method is illustrated by detection of tuberculostearic acid in a preparation of *Mycobacterium leprae* from an infected armadillo.—(Trop. Dis. Bull.)

Lugosi, L., Jacobs, W. R., Jr. and Bloom, B. R. Genetic transformation of BCG. *Tubercle* **70** (1989) 159–170.

Two substrains of BCG, the Pasteur and Japanese, were successfully transformed with *Escherichia coli*-mycobacteria shuttle plasmids, constructed from the *E. coli* plasmid, pIJ666 and the *Mycobacterium fortuitum* plasmid, pAL5000. Individual plasmids (pYUB13, pYUB14) were obtained that contain selectable antibiotic resistance markers for kanamycin and chloramphenicol resistance that can replicate in both *E. coli* and BCG. Transformation of two sub-

strains of BCG was successfully accomplished in 8/14 experiments by means of electroporation, and assessed by the growth of kanamycin-resistant colonies. The *E. coli* plasmid pIJ666 alone was unable to effect transformation.

The results suggest that the *M. fortuitum* sequences required for transformation function as an origin of replication in BCG. The introduction, persistence and the identity of the plasmids were monitored by re-isolation from consecutive subcultures and restriction analysis. The variables associated with transformation, including the age, viability, and glycine pretreatment of BCG cultures, as well as the electroporation parameters on transformation frequencies are analyzed. Consecutive transformations of BCG with plasmid DNA isolated from a BCG transformant increased the efficiency from the level of 10^1 – 10^2 obtained with the initial library to 10^3 – 10^4 colonies/ μ g DNA with functional pYUB plasmids. The hybrid plasmids were genetically stable and maintained expression of kanamycin resistance in continuous subcultures containing kanamycin for 250 generations.

The introduction and stable expression of foreign DNA in BCG on a plasmid vector establishes a basis for the construction of polyvalent recombinant BCG vaccine vehicles expressing not only putative protective mycobacterial antigens, but also antigens for other infectious and malignant diseases.—Authors' Summary

Prabhakaran, K. and Harris, E. B. Aromatic amino acid decarboxylase of *Mycobacterium leprae*. *Med. Sci. Res.* **17** (1989) 507.

The organism contains an unusual form of the enzyme *o*-diphenoloxidase that rapidly oxidizes 3,4-dihydroxyphenylalanine (dopa) and other diphenols to quinone. Among mycobacteria, this activity was unique to *Mycobacterium leprae*. The enzyme was detected in the bacteria recovered from various human tissues, armadillo tissues, mangabey monkey, and nude mice. The bacilli did not degrade tyrosine or phenylalanine. The results presented in this report show that *M. leprae* contains an aromatic amino acid decarboxylase (EC 4.1.1.28) that is specific for dopa.—(From the Article)

Thangaraj, H. S., Lamb, F. I., Davis, E. O. and Colston, M. J. Nucleotide and deduced amino acid sequence of *Mycobacterium leprae* manganese superoxide dismutase. *Nucleic Acids Res.* **17** (1989) 8378.

In order to identify protein coding sequences on *Mycobacterium leprae* DNA we obtained a clone (Y3164), isolated from an *M. leprae* DNA library cloned in lambda gt11 that expresses *M. leprae* epitopes rec-

ognized by monoclonal antibodies raised against the organism. The 2.8 kb insert of Y3164 was used to probe an *M. leprae* cosmid library. A 3.5 kb PstI fragment that hybridized to the Y3164 insert was sequenced. The deduced amino acid sequence from one of the open reading frames (621 bps) exhibits significant homology with known Mn/Fe superoxide dismutase sequences (EC.1.15.1).—(From the Article)

Experimental Infections

Gormus, B. J., Murphey-Corb, M., Martin, L. N., Zhang, J.-Y., Baskin, G. B., Trygg, C. B., Walsh, G. P. and Meyers, W. M. Interactions between simian immunodeficiency virus and *Mycobacterium leprae* in experimentally inoculated rhesus monkeys. *J. Infect. Dis.* **160** (1989) 405–413.

Thirty-four rhesus monkeys were inoculated with *Mycobacterium leprae* inoculum isolated from sooty mangabey monkeys with leprosy. Later it was learned that one of the *M. leprae*-donor mangabeys was asymptotically infected with simian immunodeficiency virus (SIV). Thus, five of the rhesus monkeys were coinoculated with *M. leprae* and SIV. Three of the five became SIV-positive and developed signs of leprosy and an AIDS-like illness. Two animals remained healthy. The coinoculated leprosy-positive rhesus monkeys developed leprosy despite serologic response patterns to *M. leprae* antigens that usually indicate leprosy resistance. Three (60%) of the five SIV-positive rhesus monkeys developed leprosy compared with 21% of the animals who received SIV-free *M. leprae* inocula. Diminished lepromin skin-test responses and de-

creasing T-helper cell percentages were observed in SIV-coinoculated rhesus monkeys with leprosy. These observations suggest that SIV increases the susceptibility of rhesus monkeys to leprosy, possibly related to loss of T-helper cell function.—Authors' Abstract

Suarez Moreno, O. and Rodriguez Silveira, J. [Effect of the suppression of the popliteal ganglion in the reproduction of *Mycobacterium leprae* in the foot pad of mice. Preliminary report.] *Rev. Cubana Med. Trop.* **39** (1987) 17–22. (in Spanish)

Ten mice were submitted to the extirpation of the popliteal ganglion of the foot pad for its further inoculation with *Mycobacterium leprae*, in order to try to eliminate the "first immunologic barrier" to be faced by the mycobacterium. Twenty-one days after the operation, this group and a control group comprising 10 normal animals were inoculated. After 8 months, the bacilli were harvested and a statistically significant difference, for $p < 0.01$, with more bacilli in the foot pads of the operated animals was found.—Authors' English Summary

Epidemiology and Prevention

Abel, L., Mallet, A., Demenais, F. and Bonney, G. E. Modeling the age-of-onset function in segregation analysis: a causal scheme for leprosy. *Genet. Epidemiol.* **6** (1989) 501–516.

Several methods have been proposed to take into account the variable age of onset of a disease in genetic analysis. A different approach is presented from an etiological point of view. To illustrate the method, we

used leprosy, an infectious disease with a variable age of onset depending on both the time of contamination with the bacillus and the latency of the disease; the role of a major gene in the susceptibility to this disease has been recently detected. The age-of-onset function was modeled to account for the two temporal processes: contamination event and incubation period. For genetic analysis, this function was combined with the probability of being susceptible to the disease, which was expressed by the use of regressive models. To test this new approach, ten sets of 500 nuclear families were simulated considering different hypotheses of contamination risks, which were either constant or dependent on contacts with contagious leprosy patients, and varying the extent to which the disease is heritable. Analyses of these data using two versions of the model indicate that the model can detect familial correlations in variable age of onset and discriminate between the different simulated effects.—Authors' Abstract

Al Sogair, S. M., Ellahbadi, S. L. and Namnyak, S. S. Leprosy in the Eastern Province of Saudi Arabia. *Saudi Med. J.* **10** (1989) 48–53.

Writing from Dammam, Saudi Arabia, these authors describe their findings in 185 consecutive new cases of leprosy diagnosed between 1983 and 1986. There were 71 Saudis and 114 non-Saudis (the latter included 88 from South India and smaller numbers from Sri Lanka, Thailand, The Philippines, Yemen, Pakistan, Bangladesh, Korea and Indonesia). The prevalence in 1986 was 4.1/100,000 for Saudis and 126.5/100,000 for non-Saudis. All confirmed patients were treated with dapsone monotherapy. Follow-up in Saudi patients was satisfactory but foreign patients were "lost to follow-up when they returned home." It is concluded that leprosy is rare in the Eastern Province of Saudi Arabia and that imported leprosy constitutes a real public health hazard to the indigenous population.—A. C. McDougall (*Trop. Dis. Bull.*)

Cestari, T. F., Ferreira, J. and Loureiro, R. [Epidemiology of leprosy in Rio Grande do Sul State, Brazil.] *An. Bras. Dermatol.* **64** (1989) 271–274. (in Portuguese)

The authors present the epidemiological situation of Hansen's disease in Rio Grande do Sul State, Brazil, analyzing the following parameters: prevalence and incidence by clinical form, sex and age. Data concerning the period 1942–1988 are remarked. They also analyze the endemy trends, relating its variations with operational factors as criteria of diagnosis and methods of control.—Authors' English Summary

Desfontaine, M., Somse, P., Baquillon, G., Kanda, P. and Limbassa, J. [Polychemotherapy of leprosy in the Central African Republic in 1987.] *Acta Leprol. (Genève)* **6** (1988) 47–55. (in French)

The Central African Republic, which once had the highest Hansen's disease prevalence rate in the central African states, had exemplary results in the control carried out on the basis of sulfonic monotherapy since 1958.

In 1983, a cluster sample survey in Upper-Sangha seemed to show that the prevalence of the disease was underestimated. It then became necessary to adopt a new national strategy whose objective would be to reduce the prevalence of leprosy in the country by 50% within 5 years. For this, a "National Programme for the Control of Leprosy in C.A.R." has been developed; in part it foresees the setting up of polychemotherapy for patients. These treatment protocols should insure healing of paucibacillary forms within 6 months and of multibacillary forms within 24 months. The new strategy of screening and decentralized treatment required retraining personnel and combining health education at individual and collective levels. The preliminary results of a national survey for the evaluation of the prevalence of leprosy are presented.—Authors' English Summary

Leprosy surveillance in England and Wales: 1951–1988. *Wkly. Epidem. Rec.* **26** (1989) 200–202.

Leprosy was made a notifiable disease in England and Wales in 1951 and a central register has been kept since that year, initially by the Ministry of Health, then by the Department of Health and Social Security and after 1982 by the Communicable Dis-

ease Surveillance Centre (CDSC). From 1966 information about cases on the register has been updated annually. Cases are removed from the register when they leave the country, die, or when cured. In 1988 the register was entered onto a microcomputer and a review of the notified cases was carried out.

There were 1221 registered cases of leprosy between 1951 and 1988 in England and Wales. After registration of an initial backlog of cases in 1951 and 1952, the annual number of notified cases rose to a peak in 1964 and has since declined. A total of 35 cases of leprosy have been notified during the last 3 years, 11 in 1986, 11 in 1987 and 13 in 1988.

Information on ethnic groups was available for 1082 cases. The most common ethnic groups were Asian, accounting for 519 cases.

There have been no indigenously acquired infections reported in this 38-year period. Clinical type was stated for 1159 cases, and comprised 345 lepromatous, 393 borderline and 382 tuberculoid cases. A small number were classified as indeterminate (18) or primary neural types (31).

A total of 894 cases have been removed from the register. Of these, 475 patients were cured, 128 died, and 291 left the country. Ninety cases were lost to follow up. Of the 237 patients remaining on the register at the end of 1988, 133 were still on antileprosy therapy and 104 remained under clinical surveillance only.

Over one half (55%) of cases currently on the register were residents of the four Thames Health Regions, and Yorkshire, Trent, West Midlands, Mersey and North Western Regions accounted for another 33%.—(*From the Article*)

Liu, G. [Implementation of MDT for leprosy in Guizhou Province.] *China Lepr. J.* 5 (1989) 66–68. (in Chinese)

Guizhou Province has signed in 1985 with WHO a 5-year collaborative leprosy control program with MDT. Guizhou has a comparatively high prevalence of leprosy (1985 = 0.24/1000) and the patients live widely separated. We have successfully implemented MDT (WHO 1982) in 2037 active cases, of which 89 are multibacillary. The

program may be divided into the following phases: A. Preparatory phase: 1) Health education to the public and the different categories of health personnel; 2) Training of field workers in the three-tiered primary health system in standardized methodology and assignment of responsibilities; 3) Active case-finding, confirmation of diagnosis, and completion of case histories. B. Implementation phase: 1) To ensure drug delivery and supervision (96.7%); 2) To check on regularity (97.4%); 3) To give immediate care to patients with indications of leprosy and drug reactions or admission to the sub-district general hospitals, if necessary; 4) Prevention of disabilities at monthly visits.

After 10–14 months of MDT, there were 446 patients (26.8%) with marked improvement, 1081 (63.2%) with moderate improvement, 163 (9.5%) stationary and 20 (1.2%) with deterioration. The reduction of BI was in average 0.58 at the end of 10 months and 0.8 at the end of 14 months of MDT. The main side effect was gastrointestinal disturbances (1.7%).

The above experiences indicate that: 1) MDT has a high therapeutic effect; 2) MDT may be successfully implemented in a highly mountainous and difficult terrain; 3) The three-tiered primary health system is a guarantee to the success of the leprosy control program with MDT; 4) The control program should be well planned and properly implemented to avoid the occurrence of multidrug resistance.—Author's English Abstract

Lombardi, C. Evaluation of leprosy epidemiology in 12 countries of the Americas, 1980–1983. *Bull. Pan Am. Health Organ.* 23 (1989) 284–294.

To assess the leprosy situation prevailing in 12 countries of Latin America and the Caribbean in 1980–1983, the author reviewed data on eight indicators—four relating specifically to leprosy cases and four to general health conditions. On the basis of scores derived from these indicators, the 12 countries were classified into three groups wherein the leprosy endemic appeared to be declining, stationary, or increasing. Countries of the first group, where the leprosy endemic appeared to be receding, exhibited generally favorable leprosy-specific indica-

tors and general health indicators, and the findings generally agreed with those of prior leprosy-prevalence surveys. Similarly, those in the third group, where the endemic seemed to be increasing, showed generally unfavorable leprosy-specific indicators and general health indicators plus general agreement with prior leprosy-prevalence surveys. In contrast, the results obtained for the three countries where the leprosy endemic seemed "stationary" differed substantially from one country to the next—but in all cases, the "stationary" situation appeared to depend less upon a stable equilibrium than upon interaction of opposing trends.—Author's Abstract

Montaville, B. and Bourée, P. [Study of leprosy in Vanuatu.] *Bull. Soc. Pathol. Exot. Filiales* **82** (1989) 351–357. (in French)

Leprosy is commonly thought to have been introduced during the 19th century and can still be considered as a public health problem in Vanuatu [South Pacific]. From 1965 to 1984, 651 cases were notified throughout the country, with a total incidence rate of 5.85‰. The 1984 survey reported 273 active cases and 190 inactive cases, most of them were paucibacillary cases. Sex ratio M/F is 1.7. Geographical distribution is clustered into two major and two minor foci. Prevalence rate is 2.1‰. The annual incidence rate is decreasing with 21 cases in 1985 and 15 cases in 1986. Reported figures are smaller than those reported in the neighboring countries.—Authors' English Summary

Terencio de las Aguas, J. [The origin of leprosy in the Americas and its present status.] *Rev. Leprol. Fontilles* **17** (1989) 157–163. (in Spanish)

First of all, the origin of leprosy and how it reached the Iberian Peninsula is explained, together with its later extension to America after its discovery. The actual endemic in the different American countries is explained with the present tendency of emphasizing the necessity to extend multidrug therapy to all the cases to achieve its eradication in the next decade.—Author's English Summary

Wang, S., et al. [Leprosy has been basically eradicated in Ningxia Province.] *China Lepr. J.* **5** (1989) 5–7. (in Chinese)

Ningxia Province of China is an area with a few leprosy patients. In the province 86 cases of leprosy have been found totally in 1958–1986, of which 57 cases were cured and 26 died or moved away to other places, and at present only three active patients with leprosy are taking treatment. The prevalence and incidence of leprosy there have been up to the criterion of basic eradication of leprosy set by the Ministry of Public Health. The majority of leprosy patients in Ningxia were from other provinces, and in the future the control work should be reinforced in order to totally eradicate leprosy.—Authors' English Abstract

Yang, L., et al. [Prevalence and control of leprosy in large cities of China.] *China Lepr. J.* **5** (1989) 2–5. (in Chinese)

The authors briefly introduce a general view of the prevalence and control of leprosy in 23 large cities of China where the population was more than one million in 1986. Among these cities, 16 (69.6%) cities are located north of the Yangzi River, being low- or nonendemic areas of leprosy, and 7 (30.4%) are south of the Yangzi River, where leprosy is endemic. The total population of the 23 cities was 60.29 million in 1986. As leprosy control has been carried out both in large cities and in the countryside all over the country, institutions of leprosy control have been built first in large cities where leprosy is prevalent. Therefore, leprosy control and its results in the large cities are better than in other places. From 1950 through 1986, 17,070 leprosy patients were found, 16,393 (95.98%) cured, and only 686 (4.02%) active cases remained at the end of 1986 in the 23 cities. During the same period, in cities and towns where the population was less than one million together with the countryside, 452,229 leprosy patients were found, 401,059 (88.69%) cured, and 51,134 (11.31%) active cases remained. The decrease of the prevalence rate in the large cities is faster than in other places in China. Leprosy has been basically eradicated in 17 (73.9%) of the 23 large cities by 1986. The authors believe that eradication

of leprosy in the large cities will be earlier than in other places in China. The reasons for the more rapid decrease of leprosy in the large cities of China are discussed.—Authors' English Abstract

Yang, X., et al. [The effect of relapse on the basic elimination of leprosy.] *China Lepr. J.* **5** (1989) 61–65. (in Chinese)

After 1949, Guangdong Province discovered 87,216 cases of leprosy by 1975. It had the highest prevalence in all China. During the same period, 51,794 cases of leprosy were treated and cured and the actual cases were 19,925 excluding the 15,497 cases of death and migration. The incidence rate was reduced from 1.15/1000 in 1961 to 0.38/1000 in 1975.

In the past 10 years, new patients were significantly decreased in number. The number of cured patients was markedly increased, more in the last 10 years than the number of new patients found in the same period. The number of counties and cities with a decreased incidence rate was apparently increased. The morbidity rate was decreased to 0.07/1000 in 1985.

The problems of the relapse of patients cured with dapsone (DDS) are analyzed also. The relapse of leprosy is the important setback during the basic extermination program of leprosy. Their main manifestation were: a) Accumulated value of relapse rate raised. b) Almost all the relapsed patients were of the multibacillary type. c) The ratio between relapsed cases and new patients increased more in the last 5 years than in former 5 years. The counties and cities in which the ratio was in excess of 20% were increased. d) The ratio of relapse cases to presently symptomatic patients in 1985 was two times that of 1980. e) The rate of decline in incidence rate was arrested.

It is emphasized that this set-back must be resolved. Guangdong has been using the combined therapy (1982) of WHO. This is an active measure for decreasing leprosy relapse and managing DDS resistance. The acceptance rate of the combined chemical therapy has reached 77.6% since 1984. Such therapy is necessary in order to increase the therapy rate and to guarantee the quality of therapy.

This paper is the first to suggest the use of the ratio of relapse cases to new active cases, which differs from the relapse rate and it should be used as a parameter in future studies.—Authors' English Abstract

Yang, Z., et al. [Calculation of leprosy spread trend with the model of exponential function.] *China Lepr. J.* **5** (1989) 81–83. (in Chinese)

The results of fitting the prevalence of leprosy and predicting its spread with the model of exponential function in Yangzhou City and its 11 counties, Jiangsu Province, basically corresponded to the relevant indices estimated on the basis of the experiences acquired in the past. Six out of the 11 counties will possibly reach the aim of basic eradication of leprosy by 1993, four by 1996 and the remaining one could also do so by 1996 after overcoming more difficulty. But because the MDT is in widespread use there, the time of implementing basic eradication of leprosy might be moved up, and generally speaking, the prospects for basic eradication of leprosy in Yangzhou City are quite optimistic. The authors point out that the method of exponential function is more exact than those of calculation on the basis of experiences for predicting the spread of leprosy.—Authors' English Abstract

Yang, Z.-M. [Ameliorative catalytic model of age-specific prevalence of leprosy simulated by using microcomputer.] *Chin. J. Epidemiol.* **10** (1989) 40–43. (in Chinese)

This paper advances an ameliorative catalytic model to imitate age-specific prevalence rates for leprosy by using a microcomputer for eight prefectures in Jiangsu Province with analyses of results. The author considers that the distribution of age-specific prevalence of leprosy tallies with the ameliorative catalytic model. It is a curve of S shape which slowly goes up at the beginning then faster rising and reaching a plateau after the point of peak. The curve is reflected in characteristics with a longer latent period, longer course of the disease and lower incidence. Through analyzing and comparing the practical leprosy prevalence in eight prefectures with three parameters of the catalytic model, it is obvious that the

prevalence was positive correlation with parameter *a* and negative correlation to parameter *b*, *k*. Therefore parameters *a*, *b*, *k* in the two-stage catalytic model, respectively, represent the force of infection, the speed of eliminating the disease and an indicator of the effect of disease control. Cat-

alytic models may be used to simulate and analyze disease data in various periods, regions or masses for comprehensive evaluation of the force of infection, ability to eliminate disease in a population, and the effect of control programs.—Author's English Abstract

Rehabilitation

Carayon, A. [Ischemic contracture of intrinsic muscles of the hand in leprosy.] *Acta Leprol.* (Genève) **6** (1988) 57–65. (in French)

An attempt at re-evaluation of the ischemic contracture of intrinsic muscles is presented as well as a study of a mechanism identical to the Volkmann syndrome (forearm muscles), its etiopathogenic treatment (excision of infarcts) in the first stage and efficient palliative methods for the Swan neck fingers and deformity of the thumb.—Author's English Summary

Malaviya, G. N., Husain, S. and Shantagunam, P. Correction of hypermobile claw fingers in leprosy by pulley insertion procedure. *Eur. J. Plast. Surg.* **10** (1987) 148–151.

Hypermobility fingers with clawing in 14 hands were operated on from 1981 to 1985. A new muscle-tendon unit was provided to flex the metacarpophalangeal joint. The insertion was into the flexor sheath; the tendon slip was brought out through the A₂ pulley, folded back and sutured to itself. At follow-up examinations, the span of the hand and grip strength were found to be increased. Integrated finger movements occurred and a good cosmetic and functional result were achieved.—Authors' Summary

Malaviya, G. N., Husain, S. and Shantagunam, P. Correction of 'Z' pinch in ulnar paralysis of leprosy by transfer of flexor pollicis longus to extensor pollicis longus. *Eur. J. Plast. Surg.* **11** (1988) 45–48.

Twenty-one thumbs having a "Z" pinch were operated on to stabilize the metacarpophalangeal joint (MCPJ). The radial half of flexor pollicis longus (FPL) was trans-

ferred to the extensor pollicis longus (EPL) after routing the FPL tendon slip to the dorsum of the proximal phalanx volar to the axis of the MCPJ. Postoperatively, the MCPJ hyperextension was reduced in all cases. All of the hands could pick up small objects without any significant loss of power during interphalangeal joint flexion.—Authors' Summary

Mathias, T. The leprosy control programme in the People's Republic of China. *Lepr. Rev.* **60** (1989) 62–63.

This report by a medical student of her elective project at China's Leprosy Center in Guangdong Province in 1988 contrasts the achievements made in reducing the prevalence of leprosy (using mainly dapsone monotherapy) with the progress still needed toward control of disability in leprosy patients. Only 14% of the accumulated leprosy patients from 1957 to 1988 are now classified as active cases, 10 provinces (one third) in north and northeast China have achieved "basic eradication," and only 0.06% of all leprosy patients in 1986 were aged < 15 years compared with 16.00% in 1957. However, rehabilitation of disabled leprosy patients in China was implemented as a specialty as late as the 1980s, and continuing care of patients to prevent disability appears ineffective as evidenced by failure of many patients to pay attention to anesthetic areas and their lack of protective shoes, gloves or glasses. However, 10 rehabilitation centers are planned, two by 1990.—C. A. Brown (*Trop. Dis. Bull.*)

Rao, K. S. and Siddalinga Swamy, M. K. Sensory recovery in the plantar aspect of the foot after surgical decompression of posterior tibial nerve. Possible role of ste-

roids along with decompression. *Lepr. Rev.* **60** (1989) 283-287.

In leprosy, involvement of the posterior tibial nerve leads to sensory loss in the plantar aspect of the foot. As a result plantar ulcers are common and lead to deformity and disability. Restoration of plantar sensation can prevent ulcer formation. Posterior tibial decompression was done for the recovery of sensation in the plantar aspect of the foot. Seventy-two patients underwent decompression on 84 feet, 25 received steroids pre- and post-operatively. The recovery of sensation was better if surgery was done before 6 months of onset of anesthesia. Decompression along with steroids gave better results than decompression alone in patients with active neuritis especially in BT cases; whereas in BB, BL and LL cases there was no significant improvement of sensation. The results are discussed.—Authors' Summary

Thompson, D. E., Buford, W. L., Jr., Myers, L. M. and Giurintano, D. J. Advances in hand biomechanics simulation. *J. Hand Ther.* **2** (1989) 142-147.

The concepts and directions described in the previous sections are derived from the development of a hand biomechanics workstation for use by orthopedic surgeons and therapists that has been underway for the past 12 years. This collaborative effort among engineers, surgeons, therapists, and physicians seeks to use math modeling, interactive graphics, and expert knowledge to build a workstation that provides natural, intuitive tools to be used in the treatment of hands.

One goal of the project is to provide the surgeon with tools for planning and analyzing reconstructive surgical procedures and for analyzing certain functional abnormalities in hands. This new approach to planning reconstructive surgical techniques was designed to permit the surgeon to base his procedures on quantitative methods rather than on the previous subjectively based methods. These tools are being designed to permit a surgeon to know in advance what excursions would be required based on the anatomy of a specific patient and the geometry of a chosen tendon path. He would also be advised of the mechanical advantage

of the muscle at each joint it acts across and of the forces necessary for specific predetermined hand actions. In addition, the visualization of the anatomy provided by the workstation permits the surgeon to obtain new insights into the reasons for selecting specific paths for tendons.

A second major goal of the workstation is to provide the therapist with new insights into the functional corrections being sought and methods of planning and analysis of external braces, splints, and prostheses. Models of soft tissue behavior and a selection of current treatment systems must be developed and tested so that the information presented is accurate and can be used to devise acceptable therapeutic methods that are based on quantifiable measures of forces, pressures, and motions. The modeling of existing splints and other devices and their response to the forces resulting from their use will one day permit the evaluation of the efficacy of different treatments and provide a means to tailor them to the needs of specific patients.—(From the Authors' Summary)

Virmond, M., Duerksen, F. and Gonçalves, A. Report and evaluation of Brazilian experience in the rehabilitation of patients with leprosy. *Lepr. Rev.* **60** (1989) 214-220.

As part of an investigation of the rehabilitation of physically disabled leprosy patients, a report and evaluation of Brazilian experience in this area is given. After describing leprosy as a genuine and relevant public health problem in Brazil because of the numbers involved, the suffering caused and the difficulties inherent in its control, the authors emphasize the important role of physical disability in this context. The description of a project set up in 1975 shows a model based on a Reference Center as an epicenter for irradiating triggering action in five Brazilian cities. The results obtained thus far consist of 32 courses given at the Reference Center and approximately 449 surgical interventions performed by one of the authors, 93.10% of which were considered satisfactory. The need to evaluate the clinical and therapeutic procedures involved from an epidemiological viewpoint is emphasized.—Authors' Summary

Other Mycobacterial Diseases and Related Entities

Badukshanova, N. M., Brusina, L. I., Shabanov, M. A., Fadeeva, N. I., Kassirskaya, N. G., Khamidulina, K. K. and Shitova, N. A. [Comparative study of delayed hypersensitivity induced by *Mycobacterium tuberculosis* and by protein fractions isolated by them.] Zh. Mikrobiol. Epidemiol. Immunobiol. 7 (1989) 84–89. (in Russian)

The role of intracellular protein fractions, isolated from *Mycobacterium tuberculosis* virulent strains in the process of the electrophoretic separation, in the development of delayed-hypersensitivity reactions has been studied. Low reactogenicity of protein fractions has been established on the basis of the development of faint immunopathological reactions in sensitized animals. High-molecular fraction 1 may be used as a sensitive preparation for the differentiation of individual mycobacterial species.—Authors' English Abstract

Barbaro, D. J., Orcutt, V. L. and Coldiron, B. M. *Mycobacterium avium-Mycobacterium intracellulare* infection limited to the skin and lymph nodes in patients with AIDS. Rev. Infect. Dis. 11 (1989) 625–628.

Three patients with AIDS who were taking 3'-azido-3'-deoxythymidine (AZT) developed cutaneous abscesses from which *Mycobacterium avium-M. intracellulare* was isolated. Culture of multiple blood samples and bone marrow aspirate from all three patients revealed that the infection was not disseminated. This is a rare form of presentation for this infection in AIDS patients. We speculate that the antiviral drug AZT was responsible for the localization of disease.—Authors' Abstract

Barbolini, G., Bisetti, A., Colizzi, V., Damiani, G., Migaldi, M. and Vismara, D. Immunohistologic analysis of mycobacterial antigens by monoclonal antibodies in tuberculosis and mycobacteriosis. Hum. Pathol. 20 (1989) 1078–1083.

Four monoclonal antibodies (MoAbs), 60.15, 61.3, 105.10, and 2.16, directed to

different proteins of *Mycobacterium tuberculosis* were used by an indirect avidin-biotin complex peroxidase-antiperoxidase method to detect mycobacterial antigens in lung, lymph node, and joint tissue specimens of tuberculous patients. Using MoAb 60.15, which recognizes a broad range of crossreactive mycobacterial proteins with a molecular mass of 28 kilodaltons (kDa), scattered materials (mycobacterial in origin) were observed, many of which were located within phagocyte cytoplasm. With MoAb 61.3, which reacts with a 35 kDa protein present in *M. tuberculosis*, *M. africanum*, and *M. bovis*, many clumped particles similar in size and shape to acid-fast bacilli were observed within the phagocyte cytoplasm (lung tissue) and positive macrophages with lysosomes were distributed throughout the cytoplasm (bronchoalveolar lavage). The specificity of this MoAb (61.3) was confirmed by the negative staining of positive lymph-node specimens obtained from a patient infected with *M. kansasii*. MoAbs 105.10 and 2.16 bind to the cross-reactive 65 kDa heat-shock protein that is present in mycobacteria and stain scattered particles and dark clumps of bacilli within the phagocyte cytoplasm. On the basis of this study, immunohistochemical detection of mycobacterial antigens appears to be useful in establishing the mycobacterial etiology of caseating granulomas and in avoiding the false-negative results obtained by traditional staining methods.—Authors' Abstract

Collins, D. M., Gabric, D. M. and De Lisle, G. W. Identification of a repetitive DNA sequence specific to *Mycobacterium paratuberculosis*. FEMS Microbiol. Lett. 60 (1989) 175–178.

A 0.2-kb DNA sequence specific to *Mycobacterium paratuberculosis*, the causative organism of Johne's disease, was isolated from a partial genomic library. The sequence was part of a larger repetitive DNA element and was present in strains of *M. paratuberculosis* from cattle, sheep, goat, deer and also a woman with Crohn's disease but not in *M. paratuberculosis* strain 18.

The sequence was not present in strains of 19 other mycobacterial species, including 31 reference serotype strains of the *M. avium*-*M. intracellulare*-*M. scrofulaceum* (MAIS) complex, some strains of which are closely related to *M. paratuberculosis*. The sequence may be useful for developing a diagnostic test for Johne's disease.—Authors' Summary

Collins, F. M. Mycobacterial disease, immunosuppression, and acquired immunodeficiency syndrome. Clin. Microbiol. Rev. 2 (1989) 360–377. (214 refs.)

The genus *Mycobacterium* contains several important human and animal pathogens, including *M. tuberculosis*, *M. bovis*, *M. kansasii*, and *M. avium*. However, this group also contains many environmental saprophytes which bear an uncertain relationship to human disease. After nearly a century of intensive study, we still know very little about the factors responsible for the pathogenicity of the tubercle bacillus or how they assist the organism to survive within the body. Some nontuberculous mycobacteria have been recognized as potential human pathogens only since the wide-spread introduction of chemotherapeutic drugs and immunosuppressants. A few reports of disseminated disease due to *M. kansasii*, *M. avium*, and *M. intracellulare* can be found in the early tuberculosis literature, but the incidence of these infections has increased sharply with the emergence of the AIDS epidemic.

Colonization of normal mucosal membranes by environmental mycobacterial species may be relatively common in many parts of the world (especially in tropical and subtropical regions). The more virulent of these opportunistic mycobacteria may become virtual members of the commensal gut and nasopharyngeal flora, surviving within the submucosal layers for relatively long periods of time. Colonized individuals become skin-test positive to *M. avium* and *M. intracellulare* sensitins. Systemic involvement by these organisms occurs only when the normal T-cell defenses are depleted as a result of old age, radiation, chemotherapy, or an intercurrent HIV infection. The latter group of AIDS patients seem especially prone to disseminated MAC infec-

tions, suggesting the existence of some sort of causal relationship between these two phenomena. Once established within the tissues, the MAC infection may involve virtually every organ of the body (liver, spleen, kidney, central nervous system, bone marrow, and intestinal tract). The source of most of these infections is presumed to be the environment, but it has proven very difficult to demonstrate the actual infection pathway, and whether the MAC infection precedes or follows HIV exposure has yet to be established definitively.

Up to 50% of AIDS patients develop a mycobacterial infection at some stage of their disease. Two thirds of the isolates are MAC serotypes 4 and 8, 10% are *M. tuberculosis*, 8% are *M. kansasii*, and 6% are *M. scrofulaceum*. Many of these isolates show aberrant cultural characteristics, colony pigmentation, mycobacteriophage susceptibility, plasmid content, and drug resistance. The significance of these differences is presently unclear.

Large numbers of *M. avium* (up to 10^{10} acid-fast bacilli per g of tissue) have been reported in some AIDS patients. This heavy tissue load, combined with the high drug resistance of these organisms, makes effective treatment of these patients almost an impossibility. As a result, the AIDS epidemic represents a return to the prechemotherapy era for patients with systemic MAC disease, and we urgently need to develop new drugs and better treatment regimens for use in this rapidly expanding patient population.—Author's Summary and Conclusions

Conville, P. S., Keiser, J. F. and Witebsky, F. G. Mycobacteremia caused by simultaneous infection with *Mycobacterium avium* and *Mycobacterium intracellulare* detected by analysis of a BACTEC 13A bottle with the Gen-Probe kit. Diagn. Microbiol. Infect. Dis. 12 (1989) 217–219.

Simultaneous infection with *Mycobacterium avium* and *M. intracellulare* in an AIDS patient was suspected after direct analysis of two BACTEC 13A blood cultures with the Gen-Probe kit for *M. avium* complex. A mixed infection was confirmed by evaluating isolated colonies. The Gen-Probe kit may provide a simple technique for detect-

ing mixed *M. avium-M. intracellulare* infections.—Authors' Abstract

Crain, E., McIntosh, K. R., Gordon, G., Pestronk, A. and Drachman, D. B. The effect of thalidomide on experimental autoimmune myasthenia gravis. *J. Autoimmun.* **2** (1989) 197–202.

Thalidomide is reported to have immunosuppressive and anti-inflammatory effects which have led to its use in the treatment of a number of immune-mediated disorders including leprosy, prurigo, discoid lupus, and Behçet's disease. In addition, thalidomide has recently been used to prevent immunological rejection phenomena following skin and bone-marrow grafts. The immune responses in these conditions are thought to be cell-mediated. However, little is known about the effectiveness of thalidomide in suppressing antibody-mediated immune responses. In the present study, we have examined the effect of thalidomide in a model antibody-mediated autoimmune disorder—experimental autoimmune myasthenia gravis (EAMG). To induce EAMG, Lewis rats were immunized with acetylcholine receptor (AChR) purified from the electric organ of *Torpedo californicus*. Groups of rats were treated daily, either with thalidomide in excess of doses reported to prevent graft-versus-host (GVH) disease in bone-marrow-transplanted rats, or with control treatments. Our results show that thalidomide failed to inhibit AChR antibody production despite good absorption and high blood levels of the drug. This suggests that thalidomide is not likely to be generally useful in the treatment of antibody-mediated autoimmune conditions. However, the selective effect of thalidomide in suppressing certain presumably cellular immune responses, while sparing antibody production, is inherently interesting, and merits further study.—Authors' Abstract

Cynamon, M. H., Swenson, C. E., Palmer, G. S. and Ginsberg, R. S. Liposome-encapsulated-amikacin therapy of *Mycobacterium avium* complex infection in beige mice. *Antimicrob. Agents Chemother.* **33** (1989) 1179–1181.

Efficacy of liposome-encapsulated amikacin and free amikacin against *Mycobacterium avium* complex was evaluated in the beige mouse (C57BL/6J-bg^J/bg^J) acute infection model. Approximately 10⁷ viable *M. avium* complex serotype 1 cells for which the MIC of amikacin was 8 µg/ml were given intravenously. Treatment was started with encapsulated or free amikacin at approximately 110 or 40 mg/kg of body weight 7 or 14 days later. In the former experiment, treatment was given two or three times per week. In the latter experiment, treatment was given daily for 5 days. The animals were sacrificed 5 days after the last dose. Liver, spleen, and lung were homogenized, and viable cell counts were determined on 7H10 agar. An analysis of variance and subsequent Tukey HSD (honestly significant difference) tests indicated that both encapsulated and free amikacin significantly reduced viable cell counts in each of the organs compared with counts in the control group. Compared with free amikacin, encapsulated amikacin significantly reduced viable cell counts in the liver and spleen. Liposome encapsulation of an active agent appears to be a promising therapeutic approach to *M. avium* complex infection.—Authors' Abstract

Gilardini Montani, M. S., Del Gallo, F., Lombardi, G., Del Porto, P., Piccolella, E., Arienzo, F. and Colizzi, V. Limiting dilution analysis of T cell unresponsiveness to mycobacteria in advanced disseminated tuberculosis. *Med. Microbiol. Immunol.* **178** (1989) 235–244.

Peripheral blood mononuclear cells from patients with advanced disseminated tuberculosis (Dis-TB) do not respond to purified protein derivative (PPD) measured as cell proliferation, lymphokine production and interleukin (IL)-2 receptor (Tac antigen) expression. Limiting dilution analysis revealed "multi-hit" curves and low frequencies of PPD-reactive T cells in cultures of Dis-TB, and "single-hit" curves and high frequencies of PPD-reactive T cells in cultures of patients with localized form of pulmonary tuberculosis. Moreover, a strict relationship between Tac antigen expression and ability of exogenous IL-2 to enhance

bulk culture cell proliferation was observed in Dis-TB patients.—Authors' Abstract

Gilburd, B. S., Markov, A. N. and Fondaminskaya, L. D. [Assay of antibodies in blood serum of patients with pulmonary tuberculosis by using mycobacterial antigen isolated by affinity.] *Probl. Tuberk.* 7 (1989) 52–56. (in Russian)

Possible use of radio- and enzyme immunoassays (RIA and EIA, respectively) in serodiagnosis of tuberculosis with antigen BCG isolated by affinity was studied in comparison to the use of the routine antigen PPD. The specific IgG antibodies were determined in serum specimens of 150 patients with various forms of pulmonary tuberculosis and 50 healthy donors. A statistically reliable difference in the efficiency of tuberculosis serodiagnosis (high levels of specificity and sensitivity) by the RIA and EIA with using antigen BCG isolated by affinity was observed.—Authors' English Summary

Günzler, V. Thalidomide—a therapy for the immunological consequences of HIV infection? *Med. Hypotheses* 30 (1989) 105–109.

The beneficial effects of the teratogenic drug thalidomide on a variety of disorders involving the immune system have been established recently. Comparison of symptoms and immunologic abnormalities of such diseases with the acquired immunodeficiency syndrome, as well as experimentally obtained results suggest that thalidomide may be a useful agent suppressing autoaggressive reactions initiated by the human immunodeficiency virus.—Author's Abstract

Guthertz, L. S., Damsker, B., Bottone, E. J., Ford, E. G., Midura, T. F. and Janda, J. M. *Mycobacterium avium* and *Mycobacterium intracellulare* infections in patients with and without AIDS. *J. Infect. Dis.* 160 (1989) 1037–1041.

A genetic probe (Gen-Probe) was used to evaluate potential epidemiologic and susceptibility differences of *Mycobacterium avium*

complex (MAC) strains isolated from 154 patients with and without the acquired immunodeficiency syndrome (AIDS). Genetic analysis revealed that 98% of the 45 patients with AIDS harbored only *M. avium* regardless of the anatomic or geographic source of the isolate; in contrast, ~40% of MAC isolates recovered from 109 patients without AIDS were *M. intracellulare*. Most *M. intracellulare* of respiratory origin recovered from patients without AIDS were involved in infectious processes. When 95 MAC isolates (*M. avium*, N = 53; *M. intracellulare*, N = 42) were evaluated for *in vitro* susceptibility to primary or secondary antimycobacterial drugs, significant differences were noted. *M. intracellulare* was more susceptible to streptomycin, rifampin, and ethambutol than *M. avium*; the converse was true for ethionamide. The results of this study suggest potentially important differences in disease spectrum and *in vitro* susceptibility profile for *M. avium* and *M. intracellulare*.—Authors' Abstract

Jackson, R. K., Juras, R. A., Stiefel, S. M. and Hall, J. E. *Mycobacterium kansasii* in a rhesus monkey. *Lab. Anim. Sci.* 39 (1989) 425–427.

The incidence of pulmonary disease caused by "atypical" mycobacteria has been increasing gradually in the human population since the 1950s. *Mycobacterium kansasii* and *M. intracellulare* are the two organisms most responsible for this trend. A rhesus monkey was euthanatized and necropsied after reacting positive to mammalian Old Tuberculin on semi-annual testing. Histopathology demonstrated the presence of small numbers of acid-fast organisms in pulmonary lesions. Further microbiological testing identified the causative organism as *M. kansasii*.—Authors' Abstract

Jekkel, A., Csajági, E., Ilköy, E. and Ambrus, G. Genetic recombination of sporoplast fusion of sterol-transforming *Mycobacterium* strains. *J. Gen. Microbiol.* 135 (1989) 1727–1733.

Wall-deficient forms of fast-growing mycobacteria were produced in growth medium containing vancomycin and glycine, and

spheroplasts were prepared by lysozyme treatment of wall-deficient cells. Spheroplasts gave rise to recombinants with high frequency (2%–6%) when they were fused using polyethylene glycol 6000. The results demonstrated that *in vivo* genetic recombination could be used to produce genetically modified *Mycobacterium* strains with applications in transformation of steroids. Useful intermediates of steroid drug synthesis and new degradation products were obtained from sterols by selected recombinant strains.—Authors' Abstract

Katila, M.-L., Mattila, J. and Brander, E. Enhancement of growth of *Mycobacterium malmoense* by acidic pH and pyruvate. Eur. J. Clin. Microbiol. Infect. Dis. 8 (1989) 998–1000.

The growth of *Mycobacterium malmoense* is dysgonic and slow on ordinary mycobacterium media. The effect of pH and pyruvate on the growth of ten strains was studied on a modification of Löwenstein-Jensen medium. Growth appeared sooner and was more abundant at pH < 6.5. At pH 7 or higher, it was scarcely or not at all visible after 6 weeks of incubation. Pyruvate enhanced the growth of five strains that grew only poorly on glycerol-containing medium, even at acidic pH. The parallel use of both pyruvate and glycerol-containing media, pH 6 to 6.5, and an incubation period of 7 weeks or longer are recommended for the isolation of *M. malmoense* on Löwenstein-Jensen medium.—Authors' Abstract

Kochetkova, E. Y. [Clinical picture and process of pulmonary tuberculosis in new cases of elderly and senile ages isolating L-forms of tubercle bacilli.] Probl. Tuberk. 7 (1989) 29–32. (in Russian)

One-hundred-forty-two new cases of pulmonary tuberculosis of elderly and senile ages were observed clinically. In terms of the vegetating mycobacterial population, all the patients were divided into three groups. It was shown that pronounced clinical and x-ray signs of pulmonary tuberculosis were characteristic of the patients isolating only bacterial forms of the pathogen (group 1). Intensive chemotherapy of the patients of that group was the most efficient. At the

same time, lethality in that group was the highest. In the patients isolating both the bacterial forms and the L-forms of the pathogen (group 2), the clinical signs of the disease were similar to those in the patients of group 1. However, the time-course of their clinical, x-ray and laboratory indices was slower and the therapy results were less satisfactory than in the patients isolating pure cultures of tubercle bacilli. In the patients isolating only L-transformed variants of tubercle bacilli (group 3), the clinical symptoms were characterized by their latent onset and torpid course. The treatment results in the patients of that group were not sufficient.—Author's English Summary

Kostina, Z. I., Pokhodzei, I. V., Tsebekova, L. A. and Ses, T. P. [Natural killer cells of blood in patients with limited tuberculosis of the lungs.] Probl. Tuberk. 9 (1989) 22–25. (in Russian)

Activity of natural killer cells in blood of 47 new cases of limited tuberculosis of the lung was studied during their complex examination. There was a significant decrease in the activity of the natural killer cells in tuberculous patients which could be used as an additional criterion of the specific process activity.—Authors' English Abstract

Lachmann, P. J. Purified protein derivative (PPD). Springer Semin. Immunopathol. 10 (1989) 301–304.

The chemical nature of PPD and its source in *Mycobacterium tuberculosis* are still not clearly worked out. However, this apparently much degraded peptide material from *M. tuberculosis* cultures has characteristic and important immunological properties and is the best example of a "T cell hapten." In addition to its use as a diagnostic reagent for diagnosing prior exposure to *M. tuberculosis*, it is a powerful "carrier" for weak antigens and may find a role in new vaccines.—Author's Conclusions

Masaki, S., Konishi, T., Sugimori, G., Okamoto, A., Hayashi, Y. and Kuze, F. Plasmid profiles of *Mycobacterium avium* complex isolated from swine. Microbiol. Immunol. 33 (1989) 429–433.

Twenty strains of *Mycobacterium avium* complex (MAC) isolated from swine and five strains from humans were examined for drug susceptibility and plasmid content. Four strains of swine origin and two strains of human origin harbored plasmid DNAs differing in molecular weights. No relationship between plasmid contents and drug resistance was observed. Southern DNA-DNA hybridization showed that small plasmids from swine MAC strains were homologous to those from human origin at the nucleotide level.—Authors' Abstract

McMurray, D. N., Mintzer, C. L., Bartow, R. A. and Parr, R. L. Dietary protein deficiency and *Mycobacterium bovis* BCG affect interleukin-2 activity in experimental pulmonary tuberculosis. *Infect. Immun.* 57 (1989) 2606–2611.

Inbred strain 2 guinea pigs were vaccinated with *Mycobacterium bovis* BCG or were left unvaccinated. They were maintained for 6 weeks on defined, isocaloric diets containing either 30% (control animals) or 10% (animals receiving low protein) ovalbumin as the sole protein source. Animals were challenged by the respiratory route with a low dose of virulent *M. tuberculosis* H37Rv and killed 4 weeks later. Protein-malnourished animals were not protected by previous vaccination with BCG. Lymphocytes isolated from various tissues were tested *in vitro* for proliferative responses to mitogen (concanavalin A [ConA]) and antigen (purified protein derivative [PPD]), production of interleukin-2 (IL-2), and response to exogenous recombinant IL-2 (rIL-2). Protein-malnourished guinea pigs responded only weakly to PPD skin tests, and their blood and lymph node lymphocytes exhibited impaired proliferation when cultured with PPD *in vitro*. IL-2 levels were consistently low in cultures of stimulated blood and spleen lymphocytes from protein-deprived animals. BCG vaccination of nutritionally normal guinea pigs, on the other hand, induced significantly more IL-2 production by PPD- and ConA-stimulated lymphocytes. The addition of exogenous mouse rIL-2 (40 and 80 U/ml) *in vitro* to PPD-stimulated blood and lymph node cells from nonvaccinated, protein-deprived

guinea pigs resulted in no improvement of the proliferative response. Previous vaccination of malnourished guinea pigs did not consistently enhance the response of PPD-stimulated lymphocytes to added rIL-2. Dietary protein deficiency and BCG vaccination appear to modulate antigen-driven cellular immunity in animals with tuberculosis by altering the production of, and the response to, IL-2 by PPD-stimulated lymphocytes.—Authors' Abstract

Miller, E. S. and Orme, I. M. Patterns of IL-2 production and utilization in mice heavily infected with *Mycobacterium bovis* BCG reflect the phase of immunity being expressed. *Immunology* 67 (1989) 221–224.

The results shown here demonstrate that in mice heavily infected with *Mycobacterium bovis* BCG Pasteur, mitogen-induced levels of interleukin-2 (IL-2) correlate temporally with the state of immunity that is being expressed in the animal during the course of the infection. Active immunity, which is conferred by populations of both CD4⁺ (L3T4) and CD8⁺ (Lyt-2) T lymphocytes, and memory immunity, which is mediated by a population of CD4⁺ T lymphocytes, were identified and distinguished in terms of their sensitivity to cyclophosphamide therapy, their ability to passively transfer specific resistance to infection with virulent *M. tuberculosis*, and their capacity to produce and/or absorb IL-2. In this regard, concanavalin A (ConA)-stimulated L3T4⁺ and Lyt-2⁺-enriched splenocytes exhibited an apparent depression in measurable levels of IL-2 when harvested during the first 40 days of the infection, which could be explained by the subsequent observation that these T cells were capable of rapidly absorbing a known quantity of recombinant IL-2 *in vitro*. Detectable levels of IL-2 in these mitogen-stimulated supernatants began to rise after day 25, which was temporally associated with a gradual shift from active immunity, to immunity mediated by cyclophosphamide-resistant memory-T cells, which did not absorb IL-2 *in vitro*. These data indicate that fluctuations in apparent IL-2 production reflect changes in the type of immunity being expressed, rath-

er than some form of defect in IL-2 production.—Authors' Summary

Neeley, S. P. and Denning, D. W. Cutaneous *Mycobacterium thermoresistibile* infection in a heart transplant patient. *Rev. Infect. Dis.* **11** (1989) 608–611.

A cutaneous infection by *Mycobacterium thermoresistibile* occurred 3 months after cardiac transplantation near the surgical scar in a diabetic patient. The organism's growth and biochemical characteristics are described, along with extensive susceptibility data. Only two previous human infections with this organism have been reported, both pulmonary. Response to oral rifampin, ethambutol, and isoniazid was complete but slow, although the organism was isoniazid-resistant.—Authors' Abstract

Pancholi, P., Vinayak, V. K. and Khuller, G. K. Immunogenicity of ribonucleic acid-protein fraction of *Mycobacterium tuberculosis* encapsulated in liposomes. *J. Med. Microbiol.* **29** (1989) 131–138.

Immunologically potent RNA-protein extracted from *Mycobacterium tuberculosis* strain H37Ra, when entrapped in phosphatidylcholine multilamellar liposomes and injected into mice, induced both cellular and humoral immune responses. Significant protection against infection with *M. tuberculosis* H37Rv was also induced in the immunized mice, as monitored by a) higher survival rates, b) decreased viable counts of *M. tuberculosis* H37Rv in lungs, livers and spleens, c) lower lung density, and (iv) lower root specific lung weight, in comparison with a control group of unimmunized mice.—Authors' Summary

Peters, M., Schürmann, D., Mayr, A. C., Hetzer, R., Pohle, H. D. and Ruf, B. Immunosuppression and mycobacteria other than *Mycobacterium tuberculosis*: results from patients with and without HIV infection. *Epidemiol. Infect.* **103** (1989) 293–300.

Infections caused by mycobacteria other than *Mycobacterium tuberculosis* (MOTT) have often been described as common in AIDS patients. To evaluate whether infections with MOTT are specific for HIV-re-

lated immunosuppression or are also frequent in patients with immunosuppression of different etiology, data on the frequency of isolation from immunosuppressed patients with HIV infection are important. Blood, stool and urine specimens from 134 patients with non-HIV-related immunosuppression and from 55 immunocompetent subjects were examined for mycobacteria. MOTT have been isolated from one immunocompetent person but from none of the immunosuppressed patients. Since in AIDS patients an initial colonization of the gastrointestinal tract (GI-tract) with MOTT is common, GI-tract biopsy specimens from an additional 80 patients were examined microscopically and histologically for mycobacteria. Mycobacteria were not isolated from these specimens.

In the same period of time 72 AIDS patients have been examined; 7 (10%) had infections with *M. tuberculosis* whereas MOTT have been isolated from 16 (22%) of these patients. Mycobacteria have been found only rarely in immunocompetent patients and have not been isolated from patients with non-HIV-related immunosuppression. The isolation of MOTT is highly correlated with an HIV-related immunosuppression.—Authors' Summary

Peterson, E. M., Lu, R., Floyd, C., Nakasone, A., Friedly, G. and de la Maza, L. M. Direct identification of *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycobacterium intracellulare* from amplified primary cultures in BACTEC media using DNA probes. *J. Clin. Microbiol.* **27** (1989) 1543–1547.

DNA probes directed at the *Mycobacterium tuberculosis* complex and *M. avium-M. intracellulare* complex were used to identify acid-fast bacilli directly from specimens grown in BACTEC 12B bottles. Clinical specimens were inoculated directly or after decontamination into a BACTEC 12B bottle, Middlebrook 7H11 agar, and Löwenstein-Jensen medium. Conventional media were incubated at 37°C in 5% CO₂ and examined weekly for 6 weeks. Identification of isolates grown on conventional media by standard biochemicals, morphology, and growth characteristics served as the reference method for identification. BACTEC

bottles were incubated at 37°C, and a growth index was taken twice a week. When a growth index of ≥ 100 was reached, 1 ml of BACTEC 12B medium was put into each of three microfuge tubes which were centrifuged for 15 min at $15,000 \times g$. Pellets were used in hybridization reactions with an *M. tuberculosis* complex probe, an *M. avium* probe, and an *M. intracellulare* probe. The results of the hybridizations of the three probes with the same sample were compared, and the highest percent hybridization was divided by the average of the two lower hybridization values. If this value, the derived patient ratio (DPR), was ≥ 3 , then the specimen was considered positive for the organism giving the highest percent hybridization. Of the 1988 specimens cultured, the results of conventional tests for the 190 conventional culture-positive specimens were 64 *M. tuberculosis*, 61 *M. avium*, 14 *M. intracellulare*, 30 other *Mycobacterium* spp., and 25 non-acid-fast bacilli. There were four cultures that each contained two different *Mycobacterium* spp. Directly probing the BACTEC 12B sediment, at a DPR of ≥ 3 the *M. tuberculosis* probe identified 83% (53 of 64) of *M. tuberculosis* isolates, the *M. avium* probe identified 92% (56 of 61) *M. avium* isolates, and the *M. intracellulare* probe identified 86% (12 of 14) of *M. intracellulare* isolates. There were no false-positive results at this DPR level. The false-negative results from probing the sediment from the BACTEC 12B bottle could not solely be attributed to the number of organisms present, the growth index, or antimicrobial therapy.—Authors' Abstract

Prantera, C., Bothamley, G., Levenstein, S., Mangiarotti, R. and Argentieri, R. Crohn's disease and mycobacteria: two cases of Crohn's disease with high anti-mycobacterial antibody levels cured by dapsone therapy. *Biomed. Pharmacother.* **43** (1989) 295–299.

Five patients with Crohn's colitis or ileocolitis (CD) refractory to conventional therapy were enrolled in an open trial with dapsone (100 mg/day). This therapy was apparently effective in 2 out of 5 patients. In these patients we observed a clinical improvement after 1 month of therapy and, in the first patient, a complete healing of all

the cutaneous and rectal ulcers. In the two responders, antibody levels to a soluble extract of *Mycobacterium paratuberculosis* (MPSE) were significantly greater than in the three nonresponders ($p = 0.03$); in the first patient, moreover, there was a rise of 39% in antibody titers following the treatment. This rise in antibody levels, that might be expected following death of the pathogen and release of antigen, is similar to that observed after treatment of tuberculosis. Our data suggest that a mycobacterial species or another pathogen that crossreact with those of MPSE, sensitive to dapsone, may in a subset of cases be responsible for the development of CD. This is the first report of clinical cure with an agent active against specific bacterial species, associated with immunologic confirmation of a response.—Authors' Summary

Rastogi, N., Blom-Potar, M.-C. and David, H. L. Drug action against intracellularly growing *Mycobacterium xenopi*. *Curr. Microbiol.* **19** (1989) 83–89.

The intracellular growth kinetics of *Mycobacterium xenopi* was studied in the murine J-774 macrophage cell line model. During the initial 4 days of infection, the bacilli divided about every 33 hr. Electron microscopy of infected macrophages showed that bacteria inside phagosomes were surrounded by a protective electron-transparent zone (ETZ). This model was used for comparing the extracellular and intracellular activities of the following drugs: pristinamycin (PRISTINA), isoniazid (INH), clofazimine (CLOFA), rifabutin (= ansamycin; ANSA), rifampin (RIFA), streptomycin (SM), ethambutol (EMB), and five fluoroquinolones, namely, ciprofloxacin (CIPRO), ofloxacin (OFLO), pefloxacin (PEFLO), enoxacin (ENOX) and norfloxacin (NORFLO). All the drugs were tested within their obtainable serum level concentrations in man. Under these conditions, CLOFA, SM, CIPRO, and OFLO were highly active against intracellularly growing *M. xenopi*. INH and RIFA were moderately active; whereas ANSA, PRISTINA, EMB, PEFLO, ENOX, and NORFLO were only growth inhibiting. The comparison of these data with extracellular activities of the same drugs underlined the discrepancies ob-

served in test-tube drug activity evaluation and its correlation with results of chemotherapy in patients in whom the drug has essentially an intracellular bacterial killing role.—Authors' Abstract

Saito, H. and Sato, K. Activity of rifabutin alone and in combination with clofazimine, kanamycin and ethambutol against *Mycobacterium intracellulare* infections in mice. *Tubercle* **70** (1989) 201–205.

Treatment of mice for 12 weeks with clofazimine or kanamycin decreased the number of organisms from lungs, liver and spleen of mice infected with *Mycobacterium intracellulare* N-260, compared with findings seen with rifabutin or ethambutol. Treatment with various drug combinations (rifabutin-ethambutol, rifabutin-clofazimine, rifabutin-kanamycin, rifabutin-ethambutol-clofazimine, rifabutin-ethambutol-kanamycin, and rifabutin-clofazimine-kanamycin), particularly in the presence of clofazimine, enhanced elimination of the organisms from these organs.—Authors' Summary

Salfinger, M., Reller, L. B., Demchuk, B. and Johnson, Z. T. Rapid radiometric method for pyrazinamide susceptibility testing of *Mycobacterium tuberculosis*. *Ann. Inst. Pasteur Microbiol.* **140** (1989) 301–309.

Pyrazinamide (PZA) is one of the most important drugs in modern chemotherapy of tuberculosis. Since PZA is active only at an acid pH, testing the susceptibility of *Mycobacterium tuberculosis* to PZA is difficult and time-consuming. Therefore, we evaluated the BACTEC system for rapid testing of PZA susceptibility at pH 6. A total of 91 *M. tuberculosis* strains and 2 different strains of *M. bovis* BCG were screened for susceptibility to PZA. Each strain was tested in special 7H12 broth supplemented with polyoxyethylene stearate containing 25, 50 and 100 µg PZA/ml. Strains resistant to 100 µg/ml were retested against 25–100 µg/ml and at an extended range of PZA concentrations from 200–6400 µg/ml. The MIC was determined with all strains within 4–20 (mean 7) days. Of the 77 susceptible strains, based on the pyrazinamidase test, MIC were ≤ 25

µg/ml for 34 strains, 50 for 38 and 100 for 2 strains. Three pyrazinamidase-positive strains had still higher MIC, 1 at 800 and 2 at 3200 µg/ml. PZA-resistant strains had MIC of 800 or greater. Monoresistance to PZA has not been detected to date. The clear bimodal distribution of MIC in this method could enable the routine clinical microbiology laboratory to perform PZA susceptibility testing as easily as the four drugs now tested in the BACTEC system.—Authors' Summary

Schulze-Röbbecke, R. and Fischeder, R. Mycobacteria in biofilms. *Zentralbl. Hyg.* **188** (1989) 385–390.

The objective of this study was to elucidate the role of biofilms as the habitat of aquatic mycobacteria. Investigations were carried out on a biofilm which grew on the inner surface of a silicone tube constantly perfused by water of a distribution system known to be contaminated with *Mycobacterium kansasii* and *M. flavescens*. The biofilm yielded 2×10^5 cfu/cm² of *M. kansasii* and 7×10^4 cfu/cm² of *M. flavescens* after 10 months of perfusion. Microscopic examination revealed that approximately ¼ to ½ of the biofilm organisms visualized by the Ziehl-Neelsen procedure were acid-fast bacteria, most of which occurred in densely packed microcolonies. These findings indicate that biofilms are an important habitat and site for proliferation of aquatic mycobacteria. Biofilms may be an explanation for the problems of controlling mycobacterial contamination of water distribution systems by means of chemical disinfection.—Authors' Abstract

Silbaq, F., Levy, L. and Bercovier, H. Doubling time of *Mycobacterium lepraemurium* in mouse footpads. *Res. Microbiol.* **140** (1989) 43–50.

The doubling time of *Mycobacterium lepraemurium* (MLM) was measured in CBA/Ca mice. In eight experiments, 5×10^3 MLM were inoculated into the hind foot pads of groups of mice, and the organisms were harvested from 1–45 days later. The harvested organisms were enumerated, and the doubling time was calculated assuming that MLM had multiplied without a lag phase

and that multiplication continued at a constant rate from inoculation to harvest. Simultaneously, the proportions of viable organisms in the inocula were determined by inoculation of serially diluted suspensions into the foot pads of other mice, harvesting 4 months later and calculating the most probable number. MLM were observed to multiply rapidly during the first several days, and more slowly thereafter; the mean initial doubling time was determined to be 0.5 days, a value much smaller than those previously reported by other workers.—AS (Trop. Dis. Bull.)

Singh, U., Solanki, R. L., Anand, V. K. and Ramdeo, I. N. Serum glycoproteins in pulmonary tuberculosis. *Indian J. Med. Res.* **89** (1989) 255–260.

Serum glycoprotein levels were studied in 50 patients of pulmonary tuberculosis and 25 healthy controls. Ten of the tuberculosis patients had stage I disease, 24 stage II, 9 stage III, and 7 had pulmonary tuberculosis with effusion. The serum levels of protein-bound hexose, protein-bound hexosamine, protein-bound fucose, and protein-bound sialic acid were significantly higher ($p < 0.001$) in patients with pulmonary tuberculosis as compared to controls; the mean values being 195.6, 189.1, 16.3 and 120.8 mg/dl, respectively, in patients and 117.0, 84.1, 7.7, and 67.2 mg/dl, respectively, in controls. The levels were not influenced by age and sex of the subjects in both groups. The levels were found to increase in all the stages of pulmonary tuberculosis with a marginal difference. Treatment with anti-tuberculosis drugs resulted in a decrease in ESR and the various fractions of serum glycoproteins at subsequent follow up. However, the mean levels did not reach those of control subjects and were not influenced by the type of therapy.—Authors' Abstract

Singhal, M., Banavalikar, J. N., Sharma, S. and Saha, K. Peripheral blood T lymphocyte subpopulations in patients with tuberculosis and the effect of chemotherapy. *Tubercle* **70** (1989) 171–178.

Peripheral blood T lymphocytes and their subsets were determined in 30 patients with pulmonary tuberculosis (15 smear positive

and 15 smear negative) and 11 healthy individuals. All patients were assessed clinically, radiologically, bacteriologically, and by tuberculin testing, and their lymphocyte counts were repeated after completion of 3 months' chemotherapy. The mean ratio of putative helper/suppressor-T-cell subsets (CD4/CD8) of healthy individuals was 1.8 (range 1.1–2.5). These ratios were independent of tuberculin reactivity and were unaffected by BCG vaccination. In both smear-positive and smear-negative patients there was a reduction in the total T-cell and CD4 counts and an increase in the CD8 count, with a concomitant reduction in the CD4/CD8 ratio. Following successful chemotherapy, the mean CD4/CD8 ratios reverted from 0.82 to 1.57 in smear-positive patients and 0.88 to 1.52 in smear-negative patients, these being near normal values.—Authors' Summary

Sritharan, M. and Ratledge, C. Co-ordinated expression of the components of iron transport (mycobactin, exochelin and envelope proteins) in *Mycobacterium neoaurum*. *FEMS Microbiol. Lett.* **60** (1989) 183–186.

Mycobacterium neoaurum was grown with a range of iron concentrations from 0.01 to 4.0 $\mu\text{g/ml}$. Synthesis of the extracellular siderophore, exochelin, the intracellular iron storage compound, mycobactin, and the iron-repressible envelope proteins were coordinately expressed. All three components of the iron transport system were synthesized when low amounts of iron (0.01 to 0.2 $\mu\text{g/ml}$) were added to the medium and were repressed when the iron concentration was increased to 0.5 $\mu\text{g/ml}$ and above. These results reinforce the conclusion that the iron-regulated proteins do fulfill an essential function in iron metabolism.—Authors' Summary

Stainsby, K., Mahmood, K. H. and Stanford, J. L. Antibodies to mycobacteria in healthy and tuberculous badgers from two English counties. *Tubercle* **70** (1989) 287–292.

Sera obtained from two groups of badgers removed in bovine tuberculosis control operations have been examined for antibodies

to 11 species of mycobacteria. From animals without post-mortem evidence of tuberculosis, levels of antibodies to mycobacteria were found to increase with age, and different patterns of antibodies were found in animals coming from two different places. Some animals (5 out of 60) without evidence of progressive infection had antibodies suggesting contact with tubercle bacilli. Animals found to have tuberculosis at post mortem had increased levels of antibody to common mycobacterial antigen, as do humans with that disease. Only 2 of the 12 tuberculous animals had markedly more antibody binding to tuberculin than to the other reagents. There was no evidence of greater specificity of antibody binding than was shown by sera of healthy badgers. The suggestion is made that contact with environmental mycobacteria might be a major factor determining distribution of tuberculosis among badgers.—Authors' Summary

Tomioka, H., Sato, K., Saito, H. and Yamada, Y. Susceptibility of *Mycobacterium avium* and *Mycobacterium intracellulare* to various antibacterial drugs. Microbiol. Immunol. 33 (1989) 509–514.

Mycobacterium avium and *M. intracellulare* of human and natural sources, identified by the Gen-Probe® Rapid Diagnostic System for *M. avium* complex (MAC), were studied for susceptibility to eight different drugs. In the case of human isolates of MAC, the following was noted. *M. avium* showed nearly the same susceptibility to streptomycin, kanamycin, ethambutol, and clofazimine as was seen with *M. intracellulare*. *M. avium* was much more resistant to rifampin and rifabutin than was *M. intracellulare*, and *M. avium* was more susceptible to quinolones, such as ofloxacin and ciprofloxacin. Conversely, in the case of MAC from natural sources, there was no difference between the susceptibility of *M. avium* and *M. intracellulare* to these antibacterial agents.—Authors' Abstract

Truffot-Pernot, C., Lecoœur, H. F., Maury, L., Dautzenberg, B. and Grosset, J. Results of blood cultures for detection of mycobacteria in AIDS patients. Tubercle 70 (1989) 187–191.

Since the advent of the acquired immunodeficiency syndrome (AIDS), numerous *Mycobacterium avium-intracellulare* disseminated infections have been recognized. Blood culture is a convenient method for diagnosing these infections. At Pitié-Salpêtrière hospital, AIDS patients with persistent unexplained fever each had three blood cultures. The blood samples, taken on three consecutive days without taking into account fever peaks, were collected in the Isolator-10 lysis-centrifugation system and inoculated onto Löwenstein-Jensen medium with and without 0.25% sodium pyruvate. From February 1986–September 1987, 564 samples taken from 165 patients were cultivated for the detection of mycobacteria. Sixty-one (10.8%) taken from 19 patients (11.5%) were positive. *M. avium-intracellulare* was the most frequently isolated mycobacterial species. In 10 patients, the positive blood culture was the only or the first positive culture for mycobacteria.—Authors' Summary

Tsoufha, G., Rook, G. A. W., Bahr, G. M., Sattar, M. A., Behbehani, K., Young, D. B., Mehlert, A., Van Embden, J. D. A., Hay, F. C., Isenberg, D. A. and Lydyard, P. M. Elevated IgG antibody levels to the mycobacterial 65-kDa heat shock protein are characteristic of patients with rheumatoid arthritis. Scand. J. Immunol. 30 (1989) 519–527.

We have previously demonstrated raised levels of IgG and IgA antibody to the mycobacterial 65-kDa heat-shock protein (hsp) in the sera of patients with rheumatoid arthritis (RA). We have now attempted to determine whether this phenomenon is specific for RA, and whether it is seen only with the mycobacterial homologue of this particular hsp gene family. We therefore screened antibody levels to the mycobacterial and *Escherichia coli* hsp65, and the mycobacterial, *E. coli*, and human hsp70, in sera from RA, systemic lupus erythematosus (SLE), tuberculosis (TB), ankylosing spondylitis (AS), Crohn's disease, and control donors. RA sera show the greatest increase in IgA binding to the mycobacterial hsp65, but no increase in IgA binding to the *E. coli* homologue. Similarly, only RA and

TB sera show increased IgG binding to the mycobacterial hsp65, and we have shown previously that the titer is greater in RA. In contrast, the use of mycobacterial and *E. coli* hsp70 preparations as control bacterial hsp gene products has shown that RA patients do not differ from TB or SLE patients in their antibody binding to these proteins. Moreover, neither IgA nor IgG antibody to the human hsp70 in RA sera were higher than in TB, and the IgA binding was not higher than in SLE. These findings suggest that elevated IgG antibody levels to the mycobacterial hsp65 show some disease specificity, and further studies with the human homologue and at the T-cell level are required.—Authors' Abstract

Wallace, R. J., Jr., Bedsole, G., Sumter, G., Sanders, C. V., Steele, L. C., Brown, B. A., Smith, J. and Graham, D. R. Activities of ciprofloxacin and ofloxacin against rapidly growing mycobacteria with demonstration of acquired resistance following single-drug therapy. *Antimicrob. Agents Chemother.* **34** (1990) 65–70.

The susceptibility to ciprofloxacin of 548 clinical isolates of rapidly growing mycobacteria belonging to eight subgroups or species was determined. The 170 isolates of *Mycobacterium fortuitum* biovar. *fortuitum* were most susceptible; the MIC for 90% of the organisms was 0.125 µg/ml. The other biovariants of *M. fortuitum*, *M. smegmatis*, and the *M. chelonae*-like organisms were less susceptible; the modal MIC was 0.5 µg/ml, and the MIC for 90% of organisms was 1.0 µg/ml. The two subspecies of *M. chelonae* were generally resistant, with only 8% of 206 isolates falling in the moderately susceptible category (MIC, 2 µg/ml) and only 2% falling in the susceptible category (MIC, ≤ 1 µg/ml). MICs of ofloxacin averaged 1 to 2 dilutions higher than those of ciprofloxacin for all subgroups tested. Three patients with *M. fortuitum* cutaneous disease relapsed after an initial response to therapy with ciprofloxacin, and their isolate was shown to have acquired drug resistance. Mutational frequencies for *M. fortuitum* with ciprofloxacin were relatively high (10^{-5}

to 10^{-7}), and MICs for single-step mutants were similar to those for the clinically resistant strains. Thus, despite the excellent activity of ciprofloxacin against rapidly growing mycobacterial groups other than *M. chelonae*, single-drug therapy should be used with caution because of the risk of development of mutational resistance.—Authors' Abstract

Woodbury, J. L. and Barrow, W. W. Radiolabelling of *Mycobacterium avium* oligosaccharide determinant and use in macrophage studies. *J. Gen. Microbiol.* **135** (1989) 1875–1884.

Internal radiolabeling procedures were used to radiolabel the oligosaccharide determinant of the glycopeptidolipids (GPL) from serovars 4 and 20 of the *Mycobacterium avium* complex. Mycobacteria were cultured in the presence of [6-³H]fucose, [2-³H]mannose or [methyl-³H]methionine, after which radiolabeled native lipid was extracted and distribution of radioactivity in native and deacetylated lipid was determined by thin-layer chromatographic methods. Incorporation of radiolabel was confirmed by examining acid hydrolysates of purified GPL for ³H-labeled sugars on cellulose thin-layer plates. Least incorporation of radiolabel into GPL was observed with [6-³H]fucose; whereas better incorporation was obtained with [2-³H]mannose and [methyl-³H]methionine. Use of [methyl-³H]methionine resulted in the radiolabeling of the methylated sugars in both the oligosaccharide determinant and the 3,4-di-O-methylrhamnose located at the terminus of the peptide core. Use of [2-³H]mannose resulted in the incorporation of radioactivity into the oligosaccharide determinant as 2-O-methylfucose, found in the GPL of both serovars 4 and 20. GPL radiolabeled with [2-³H]mannose were subsequently examined in macrophage cultures and found to be relatively inert to degradation by those phagocytic cells. These results substantiate earlier findings with the GPL of serovar 20 and indicate that these mycobacterial components may play a role in pathogenesis.—Authors' Abstract