CURRENT LITERATURE

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

General and Historical

Bechelli, L. M., Garrido Neves, R., Hahn, M. D., Haddad, N., Pagnano, P. M. G. and Melchior, E. Etude comparative clinicohistopathologique des classifications de la lèpre de Madrid et de Ridley-Jopling. [Comparative clinicohistopathological study of Madrid and Ridley-Jopling classifications of leprosy.] Acta Leprol. (Genève) **89** (1982) 1–15. (in French)

For the purpose of considering the practicability of using the Ridley-Jopling (R-J) classification in leprosy control programs, a comparative study of it with the Madrid classification was undertaken with the participation of two pathologists and three leprologists. These ignored the histological classification and those the "clinical" one. The pathologists have utilized both classifications being uninformed about the diagnosis of the other colleagues; and each of them did not know how he had himself classified the cases either by the Madrid or by Ridley and Jopling (R-J) classification.

The conclusions were as follows:

1. There was a great agreement, sometimes almost complete, between the two histopathologists when the Madrid classification was adopted. The discordance was evident with the R-J classification, with exclusion of the indeterminate cases.

2. For the leprosy cases I (LI), T (TT) and Tr (BT) there was a great agreement between the three leprologists and the two histopathologists utilizing the Madrid and R-J classification, especially for the first one and for the senior pathologist. The difference was evident for lepromatous leprosy in the R-J classification, for the junior pathologist.

3. The senior pathologist had a greater agreement with himself when the results of the Madrid classification and those of R-J were compared.

4. For leprosy control the classification should be as simple as possible and also have a sound scientific basis to allow an evaluation of epidemiological findings and other aspects. The existence of a greater number of forms or groups in the R-J classification make its utilization more difficult in the field, including for the pathologists, and at the same it does not offer advantages under the epidemiological and control points of view.

5. The use of the R-J classification in leprosy control programs faces several difficulties. Even correctly applied, from prophylactic point of view, there is no advantage in differentiating the cases BL (a), BL (b) and LL: for all these patients the treatment and surveillance should be strict because they are the main patients responsible for the dissemination and maintenance of the endemics.—Authors' Summary

Haddad, N., Bechelli, L. M., Garrido Neves, R., Hahn, M. D., Melchior, E., Jr. and Pagnano, P. M. G. Analyse de la concordance et de la discordance inter- and intrapathologistes dans une étude comparative des classifications de la lèpre de Madrid et de Ridley-Jopling. [Analysis of the inter- and intrapathological concordances and discordances in a comparative study of Madrid and Ridley-Jopling classifications of leprosy.] Acta Leprol. (Genève) **89** (1982) 17–26. (in French)

Eighty-six patients of leprosy have been examined by three leprologists; they have been classified according to the Madrid classification and their lesions biopsied and sent to two pathologists for independent histopathological examination. The pathologists have not received any information regarding the cases. Firstly the "senior" pathologist (A) utilized the Madrid classification and the "junior" pathologist (B) the Ridley-Jopling classification. In a second phase, the "senior" pathologist utilized the Ridley-

Jopling classification and the "junior" pathologist the Madrid classification. Both pathologists did not know their own previous histopathological diagnosis at the second phase.

The analysis of concordance and discordance between the histopathological diagnosis of the two pathologists and for the same pathologist, on utilizing the two classifications, have elicited the following conclusions:

1. There has been more concordance between the two pathologists on utilizing the Madrid classification than the Ridley-Jopling classification for the lepromatous, borderline and tuberculoid in reaction patients.

2. The comparison of the two classifications for each pathologist on "blind" examination of the material, has shown more concordance for the "senior" pathologist than for the "junior" pathologist.

3. These observations have led to the final conclusion that the Ridley-Jopling classification brings some difficulties to the pathologists with limited experience in leprology and therefore it should be utilized only by experienced pathologists.

4. This preliminary study shows the necessity of performing another one with larger number of patients, in the active phase, with larger number of pathologists, preferably from different countries, and by utilizing the same methodology of "blind" examination of the material.—Authors' Summary

Hendrick, S. S. and Wilkin, J. K. Leprosy. Am. Fam. Physician **26** (1982) 161–166.

The incidence of Hansen's disease is increasing in the United States. Early diagnosis can prevent irreparable damage. Sensory changes are often the first manifestations of tuberculous leprosy. In the lepromatous form, a variety of skin lesions pose diagnostic puzzles. The definitive diagnosis is made by histopathologic examination of skin or mucosa. Multidrug therapy, specifically dapsone and rifampin, is recommended for all newly diagnosed leprosy patients. In cases of untreated borderline or lepromatous leprosy, dapsone prophylaxis is advised for family contacts under 25 years of age.—Authors' Abstract

Pattyn, S. R. Mouse foot pad technique for evaluation of drug resistance of *M. leprae* and other laboratory techniques to be used in leprosy control programmes. Acta Leprol. (Nouvelle série) **1** (1983) 29–32.

There is no need for mouse foot pad facilities for drug sensitivity testing to start leprosy control programs since the size of the problem is known. There will however be a need for some mouse foot pad work in later stages to document relapse cases and test relapse strains for drug sensitivity. Arrangements should be made to ship such specimens to existing laboratories.

There is more need however for facilities for monitoring drug intake. Finally, where nonexistent, there may be incidentally some need for quality control of imported drugs, which can be solved through collaboration with existing facilities in other countries.—Author's Summary

Stingl, P. Leprosy. Classification—clinical picture—diagnosis—treatment. Z. Hautkr. **57** (1982) 1559–1567. (in German)

This paper reports on the causative organism of *Mycobacterium leprae,* its mode of transmission, its classification into polar and interpolar forms, its diagnostic methods including skin smear, skin and nerve biopsy, as well as on its treatment.

The leprosy reaction presents a main complication of leprosy and can be considered as an emergency. Its pathomechanism, symptoms and treatment are mentioned.—Author's Summary

Chemotherapy

Balakrishnan, S., Seshadri, P. S., Neelan, P. N., Venkataramaniah, H. N., Harikrishnan, S. and Bhatia, V. N. Studies on sulphone resistant strains of *M. leprae* in field and institutionalized cases of leprosy. Lepr. India **55** (1983) 71–73.

The proportions of dapsone-resistant strains of *M. leprae* in cases from the field and in patients institutionalized for treatment were compared. Identification of dapsone resistance was done by mouse foot pad experiments carried out in the laboratory in Central Leprosy Teaching and Research Institute, Chengalpattu. A higher percentage of resistance was detected among institutional patients as compared to field cases. The distribution of M.I. ranges of *M. leprae* in dapsone-resistant subjects was not different from that in dapsone-sensitive cases.—Authors' Summary

Goihman-Yahr, M. Leprosy, an overview. Int. J. Dermatol. **21** (1982) 423–431.

Leprosy is both a public health problem and a model for host-parasite relationship. There is much progress in many areas, and in most, hurdles are not only scientific but also social and financial. The following are goals to reach: 1) progress of growth of *Mycobacterium leprae* in animals (to be able to breed armadillos in captivity or to substitute them with other animals); 2) to develop methods for *in vitro* culture of *M. leprae*; 3) to obtain antigens from *M. leprae* and characterize them immunologically; 4) to achieve techniques for objective diagnosis of infection with *M. leprae* and of leprosy (these techniques will most likely be serologic); 5) to develop methods to direct and manipulate immune response. This should include a better definition of immunologically active cells, their characterization by reliable methods, and an understanding of their functions. Immune control mechanisms and ways to channel them should also be understood; and 6) to have better methods of treatment including flexible adaptable schedules.

It will be clear that "solving" the problem of leprosy requires "solving" the social problems in developing countries, as well as unraveling the mystery of the precise control of immune response. As I pointed out, the problems that we face are basically similar to those of autoimmune disorders and of cancer immunology. Research in autoimmune diseases or cancer might provide answers to questions posed by leprosy. It may also be that research in leprosy could supply answers to questions concerning cancer and autoimmunity.—Author's Summary

John, J. K., Pannikar, V. K., Verghese, A. and Christian, M. Social and personality factors in dapsone resistant patients. Lepr. India **55** (1983) 100–106.

A preliminary study in which a sample of 25 patients registered with the Schieffelin Laboratory Research and Training Centre, Karigiri, India, with proved secondary DDS-resistant leprosy were compared with 25 patients not suspected to be DDS resistant for social and personality factors which could have led to noncompliance in treatment.

A structured interview schedule enquiring into factors leading to irregular treatment and the psycho-social background of the patient was used. The Eysank's Personality Inventory (EPI) and personality traits assessment on a linear analogue was also administered to them.

Statistical analysis showed that DDS-resistant leprosy patients were, as a group, more irregular for treatment, showed personality traits of being casual and uncontrolled, and scored high on the neuroticism scale of the EPI.—Authors' Summary

Katoch, K., Ramu, G. and Ramanathan, U. Toxic epidermal necrolysis (Lyell syndrome). A case report. Lepr. India **55** (1983) 133–135.

Toxic epidermal necrolysis is a severe reaction of skin characterized by wide-spread erythema and detachment of epidermis resembling scalding. It is caused by a number of viruses, fungi, bacteria, drugs and neoplastic diseases. In the case reported here it was due to dapsone or isozone.—Authors' Introduction

Kohsaka, K., Yoneda, K., Arimochi, Y., Makino, M., Mori, T. and Ito, T. Nude mice as a model for chemotherapy of leprosy. *Proc. 1979 Third Intl. Workshop on Nude Mice.* Reed, N. D., ed. New York, NY: Gustav Fischer, 1982, Vol. 1, 59–65.

Leprosy patients were treated with 450 mg of rifampin daily, and materials for an inoculum were obtained by biopsy before and after treatment. *Mycobacterium leprae*

obtained from the patients were inoculated into the right hind foot pads of nude mice. The results of the experiments indicate that rifampin shows tremendous initial killing effect for *M. leprae*; the bacilli lost infectivity for nude mice after only two days of administration with 450 mg of the drug to man. For chemoprophylactic studies, *M. leprae* from experimental leprosy in nude mice were used, and the infected nude mice were given 0.5 mg (once) or 0.2 mg (six days a week, for two weeks) of rifampin orally. The results suggest that a single administration of 1500 mg rifampin or 600 mg daily for two weeks of rifampin may be effective as chemoprophylaxis of human leprosy. Chemoprophylactic administration with a daily dose of 0.03 mg of DDS or 0.2 mg of clindamycin for one month or 0.03 mg of minocycline for three months was not able to prevent the growth of *M. leprae* in nude mice.—Authors' Abstract

Ramu, G. and Sengupta, U. Preliminary trial of intervention levamisole therapy in persistently bacteriologically positive lepromatous leprosy. Lepr. India **55** (1983) 64–67.

The intervention treatment with the immuno-adjuvant levamisole was given in 14 persistently bacteriologically positive lepromatous cases who had had adequate chemotherapy for over five years. There was a temporary conversion of the early lepromin reaction in a majority of the patients with a corresponding improvement in clinical and bacteriological status. However, the lepromin response was short lived. Most patients had become bacteriologically negative at the end of one year. No adverse effects due to levamisole therapy were encountered.—Authors' Summary

Ree, G. H. The treatment of leprosy in Papua New Guinea. Papua New Guinea Med. J. **25** (1982) 142.

This is a very short, one-page article advising health personnel on the drug treatment of leprosy in Papua New Guinea. It warns about dapsone resistance and draws attention to the World Health Organization's recommendations on multidrug treatment of multibacillary leprosy. Although it refers to supervised treatment of rifampin and clofazimine, it does not say how long they should be supervised and there is therefore some doubt. Otherwise, the article is helpful to field staff.—J. C. Hargrave

Revankar, C. R., Naik, S. S. and Ganapati, R. Dapsone compliance in an urban field project. Lepr. India **55** (1983) 117–121.

Available reports both from rural and urban leprosy centers prove beyond any doubt that dapsone is not being consumed regularly by all those who show regular clinic attendance. No reports are available from urban field projects where the SET program is being operated. In this study surprise urine samples of 294 patients of all types (smear positive 53%) mainly on dapsone monotherapy attending 1) leprosy treatment centers in slum clinics situated in the field areas adopted for SET work by the Bombay Leprosy Project and 2) general hospital clinics situated predominantly outside the project area were analyzed for dapsone/creatinine ratio to judge the extent of drug compliance. Two hundred one out of 294 (68%) were regular and 82 out of 294 (28%) were irregular in consuming DDS as judged by urine examination. Sixty-seven percent of smear positive cases were regular. No difference was found in regularity between patients living within the project area (intensive follow up is done in this group to remind about treatment) and outside project area patients (no reminder followup is done). Similarly no difference was observed in regularity among the patients attending slum clinics and general hospital clinics. It could be stated that facilities for treatment offered at general hospitals or dispensaries and encouraging voluntary reporting could be quite fruitful and economical for obtaining better drug compliance in urban areas.—Authors' Summary

Vellut, C., Van der Veid, D., Supplisson, C. and Decazes, J. M. L'absentéisme au cours du traitement de la lèpra: analyse des causes révélées par une enquête en Inde du Sud. [Absenteeism during treatment of leprosy: An analysis of its causes as evidenced by a survey in South India.]

Acta Leprol. (Genève) **89** (1982) 27–37. (in French)

The reasons for absenteeism during leprosy treatment were investigated in a rural area of southern India. One hundred twenty patients known as "absents" to most controls were first interviewed and the major causes for absenteeism thus determined. A questionnaire was then elaborated in view to reveal these principal causes with efficiency and was applied by eight investigators to 1200 patients, mostly absents or irregular to medical visits; 620 were selected at random for computer analysis. Results suggest that anxiety for loss of income while attending the medical control and erroneous impression of cure as soon as skin lesions have improved could be of first importance. Nevertheless no relation appears between absenteeism and income level, number of persons depending on the patient, or type of leprosy. Adverse reactions attributed to dapsone (DDS) are also frequently reported, especially fever, because of confusion with leprosy reactions, malaria and any other febrile condition. Assiduity to medical visits could be determined during the year following this study in 1191 of the 1200 patients showing clear beneficial effects over this period.—Authors' Summary

Yagnik, C. S., Jogaikar, D. G. and Mehta, J. M. Effect of levamisole on clinical outcome and DNCB conversion in leprosy patients. A long term study. Lepr. India **55** (1983) 68–70.

Fifteen patients of lepromatous leprosy within the first six months of diagnosis were studied. Seven controls received standard dapsone (DDS) treatment and placebo while eight patients received cyclical levamisole treatment (150 mg daily for three days repeated after a gap of two weeks), in addition to standard DDS treatment. Patients were followed up clinically for lepra reactions, serial B.I. and DNCB test for two years. We observed that ENL reactions were more common and more severe in the levamisole-treated group, while an upgrading type reaction occurred only in one of the control patients. B.I. remained the same in both groups throughout, while the DNCB score was higher in controls than in levamisole-treated groups. Thus, levamisole treatment does not seem to have caused stimulation of CMI in our patients as judged by DNCB reaction, while it may have caused stimulation of humoral immunity as seen by higher incidence of ENL reaction. This may be undesirable in lepromatous patients.—Authors' Summary

Clinical Sciences

Balakrishnan, S., Bhatia, V. N. and Harikrishnan, S. Hepatitis-B-surface antigen (HBsAg) in leprosy patients. Lepr. India **55** (1983) 45–48.

The detection of the presence of hepatitis-B-surface antigen (HBsAg) using indirect hemagglutination technique (IHA) was carried out in 134 lepromatous leprosy cases (LL), 22 lepromatous leprosy cases during ENL reaction (LL with ENL), 24 borderline leprosy (BL) cases, 7 borderline tuberculoid cases (BT) and 31 control subjects. The maximum percentage positivity of 32% was seen in cases with LL as compared to 6.2% in controls. The difference was found to be statistically significant ($p < 0.01$).—Authors' Summary

Bhatia, V. N., Balakrishnan, S. and Harikrishnan, S. Serological study for presence of C-reactive protein, rheumatoid factor, anti-streptolysin O in leprosy cases. Lepr. India **55** (1983) 86–90.

Serological tests for the detection of C-reactive protein (CRP), rheumatoid factor (RF) and anti-streptolysin 'O' (ASLO) antibodies employing latex test were carried out in 120 cases of active lepromatous leprosy (LL), 14 LL cases with ENL during active and subsided phases, and 25 controls. Out of 120 LL cases 25.8% gave a positive reaction to CRP and ASLO. Only 4.2% of the cases with LL were positive for rheumatoid factor. None of the controls showed positive reaction in these tests. During ENL,

100% of the sera showed positive test for CRP as against 35.7% during subsidence of reaction. Tests for RF were positive in 28.5% of the cases with active ENL as compared to none during subsided ENL. Raised ASLO titers were noticed in 38.2% cases with active ENL as against 21.4% during subsided phase of ENL.—Authors' Summary

Brandt, F., Kampik, A., Malla, O. K., Pokharel, R. P. and Wos, J. Blindness from cataract formation in leprosy, in: *Turning Points in Cataract Foundation Syndrome and Retinoblastoma (Developments in Ophthalmology,* Vol. 7). Straub, W., ed. Basel: Karger, 1981, 1–12.

Of 744 leprous patients, 61 (8.2%) had cataract-induced blindness: 46 patients (6.2%) were unilaterally blind and 15 (2.0%) were bilaterally blind. The mean age of patients with cataracts was 63.4 years in the tuberculoid-type leprosy and 56.4 years in the lepromatous type. The appearance of cataracts in lepromatous patients at an earlier age than in tuberculoid patients is significant ($p < 0.005$). Of 41 cataractous eyes with posterior iris synechiae, 42.1% were observed in patients with tuberculoid-type leprosy and 59.1% in patients with the lepromatous type. Histopathological examination of 24 lenses showed that irregularities of the lens epithelium were present in 79.2%. Posterior iris synechiae were seen in 66.7% and fibrous pseudometaplasia in 41.7%. Posterior migration of the lens epithelium in the posterior subcapsular area was observed in 54.2%. In view of these clinical and histopathological findings, we discuss the probability that most cataracts in leprous patients are complicated in nature.—Authors' Summary

Donde, S. V., Shah, A. and Antia, N. H. Nerve conduction in leprosy: *in vivo* and *in vitro* study. Lepr. India **55** (1983) 12–21.

Sensory and mixed nerve conduction studies were performed on the distal segments of the radial, sural, median and ulnar nerves of leprosy patients from the entire spectrum of the disease. The values obtained were compared with those of age-matched normal controls. All the clinically involved nerves, and about 30% of clinically normal patient nerves, showed a delayed conduction velocity. The conduction velocity difference was most significant ($p < 0.001$) for the cutaneous branch of the radial and sural nerves as compared to the ulnar and median.

Five patient nerves, that were clinically normal and showed a normal conduction velocity when tested *in vivo,* were biopsied and studied *in vitro* to determine the type of early nerve damage. C and Aδ fiber involvement was found in all these nerves. It is concluded that leprosy is a diffuse neuropathy and there is early detectable nerve involvement even in clinically normal nerves of leprosy patients.—Authors' Summary

Higashi, G. I., El-Gothamy, Z. and Habib, M. A. Immunoglobulin deposits in leprosy skin. Ann. Trop. Med. Parasitol. **77** (1983) 87–94.

An immunofluorescent study was carried out on skin biopsies from 12 leprosy patients who had been suffering from the disease for periods of one to 30 years; all were treated with dapsone and clofazimine at one stage.

Skin biopsies made from reactive nodular lesions showed that all 12 had IgG deposits and seven had IgM deposits in the dermal-epidermal junction. No IgA, IgE, C3 and fibrinogen were found deposited in any biopsy. All patients had significantly raised levels of serum immunoglobulins and rheumatoid factor. Anti-epithelial (eight patients) and anti-nuclear (three patients) antibodies were also found. The possible role of autoantibodies in the present findings is discussed.—Authors' Summary

Jain, V. K., Verma, K. C. and Aggarwal, S. S. A study of serum fibrinolytic activity in erythema nodosum leprosum (ENL). Lepr. India **55** (1983) 95–99.

Fibrinolytic activity was studied in 31 patients of leprosy and ten healthy controls. Fibrinolytic activity was found to be significantly decreased in patients with erythema nodosum leprosum (ENL), compared to those with uncomplicated lepromatous leprosy who, in turn, showed lowered activity compared to patients with the tuberculoid form of leprosy and controls. The severity

of ENL correlated very well with decrease in fibrinolytic activity. Fibrinolytic activity was improved to levels obtained in uncomplicated lepromatous leprosy after the subsidence of reaction. So it would seem reasonable to suggest that estimation of fibrinolytic activity provides a reliable criteria to quantitate the severity of erythema nodosum leprosum.—Authors' Summary

Kher, J. R., Baji, P. S., Ganeriwal, S. K., Reddy, B. V. and Bulakh, P. M. Serum lipoproteins in lepromatous leprosy. Lepr. India **55** (1983) 80–85.

Serum total cholesterol and the lipoprotein fractions were studied in 40 subjects of lepromatous leprosy and age-matched controls. The study revealed a significant decrease in serum cholesterol in the disease group as compared to control group. The decrease in cholesterol was 28.76%. An alteration in serum lipoprotein fractions was observed in disease group. The β-lipoprotein fraction showed a significant decrease along with a rise in α-lipoprotein fraction. A positive correlation was also observed between total cholesterol and β-lipoproteins. The significance of the above findings are discussed.—Authors' Summary

Pal, R., Pal, B. and Ghosh, S. Concentration of serum calcium in leprosy. Lepr. India **55** (1983) 76–79.

The difference in the concentrations of the serum calcium at intervals, i.e., after three weeks and three months, varies with two polar types of leprosy under treatment with DDS. There is no statistically significant rise of serum calcium level in lepromatous cases at intervals when under treatment. In the tuberculoid group mean serum calcium levels before treatment, after three weeks' treatment, and after three months' treatment are 8.5 mg%, 9.2 mg%, and 10.0 mg%, respectively. P-value is significant when compared with the figures observed initially and after three months. There was no change in the level of serum calcium after three weeks of treatment.—*From* the article.

Yemul, V. L., Sengupta, S. R. and Dhole, T. N. Protease inhibitors activity in lepromatous leprosy and lepra reactions. Lepr. India **55** (1983) 91–94.

Serum alpha one antitrypsin levels were measured in 50 healthy, age- and sex-matched controls with 45 lepromatous leprosy (LL) cases and 5 cases of lepra reaction (LR). It was noted that the mean level in healthy controls was 281.00 mg%, while the mean levels in LL patients was 421.00 mg% and in LR 570.00 mg%. The elevation of alpha one antitrypsin was statistically significant in LL patients. It is possible that the rise is a reaction to release of proteases and/or higher complement activity, which are the results of a high bacillary loading to formation of immune complexes.—Authors' Summary

Immuno-Pathology

Adu, H. O., Curtis, J. and Turk, J. L. Role of the major histocompatibility complex in resistance and granuloma formation in response to *Mycobacterium lepraemurium* infection. Infect. Immun. **40** (1983) 720–725.

Resistance to a subcutaneous infection with a moderate dose of *Mycobacterium lepraemurium* was investigated in C57BL/6 mice and in three congenic strains with the BALB background (BALB/c, BALB/B, and BALB/K). Resistance after ten weeks of infection was found not to be linked to the major histocompatibility complex. The ability to develop a delayed hypersensitivity response to an ultrasonicate of *M. lepraemurium* was associated with the background genes, and this ability had no influence on resistance to *M. lepraemurium*. Granuloma formation at the infection site in the early stages appeared to be linked to the H-2^b haplotype. The types of cells involved in the granulomas were also investigated.—Authors' Summary

Anthony, J., Vaidya, M. C. and Dasgupta, A. Ultrastructure of skin in erythema nodosum leprosum. Cytobios **36** (1983) 17–23.

The ultrastructural changes observed in erythema nodosum leprosum lesions were gross damage to blood vessels, particularly the endothelial cells, and deposition of immune complexes in vessel walls. Infiltrating cells and alteration of the ground substance formed a constant feature of the reaction. These changes are characteristic of an immune complex reaction.—Authors' Abstract

Bach, M.-A., Wallach, D., Flageul, B. and Cottenot, F. *In vitro* proliferative response to *M. leprae* and PPD of isolated T cell subsets from leprosy patients. Clin. Exp. Immunol. **52** (1983) 107–114.

In vitro proliferative response to *Mycobacterium leprae* and PPD of T cell subsets, isolated by selective depletion procedure from peripheral blood using OKT4 or OKT8 monoclonal antibodies plus complement, was investigated in leprosy patients. Whole peripheral blood mononuclear cells (PBMC) developed a strong proliferative response to both *M. leprae* and PPD in most tuberculoid patients. This proliferation was confined to T cells, and concerned predominantly $OKT4^+$ cells. Both antigens, however, induced a smaller, but significant proliferation of $OKT8^+$ cells. In lepromatous patients, proliferative response of whole PBMC incubated with *M. leprae* was in most cases insignificant, at variance with PPD-induced proliferation, which was not significantly lower than that of PBMC from tuberculoid patients. In a majority of *M. leprae* nonresponders, neither $OKT4^+$ nor $OKT8^+$ enriched PBMC developed a proliferative response to *M. leprae*. Unexpectedly in four *M. leprae* unreactive patients, control treatment of PBMC with complement alone restored a strong proliferative response to *M. leprae*. Taken together, these results suggest that *in vitro* unresponsiveness to *M. leprae* results, at least in some patients, from an active suppressor mechanism but that the effector phase of such suppression does not directly involve $OKT8^+$ T cells.—Authors' Summary

Bach, M.-A., Wallach, D., Flageul, B. and Cottenot, F. Monoclonal antibody-defined T-cell subsets in lepromatous leprosy. Ann. Microbiol. (Paris) **133B** (1982) 165.

Using an indirect immunofluorescence assay and monoclonal antibodies OKT3, OKT4 and OKT8—which recognize all T cells, helper T cells, and suppressor/cytotoxic T cells, respectively—the percentages of these T cell subsets present in the peripheral blood of leprosy patients were determined. Patients without recent reactions had T cell subset profiles which seemed to vary according to their bacterial load, with high percentages of suppressor/cytotoxic T cells in untreated bacillary patients and normal T cell balance in non-bacillary treated patients. Patients with recent ENL showed a T cell imbalance, with a decreased proportion of suppressor/cytotoxic T cells. In most patients, T cell subset percentages returned to normal values after the ENL episodes.—Authors' Abstract

Chakrabarty, A. K., Maire, M., Saha, K. and Lambert, P. H. Identification of components of IC purified from human sera. II. Demonstration of mycobacterial antigens in immune complexes isolated from sera of lepromatous patients. Clin. Exp. Immunol. **51** (1983) 225–231.

Immune complexes have been purified from sera of patients with lepromatous leprosy, using solid phase conglutinin and analyzed by SDS-PAGE. Some of their components have been immunologically identified after electrophoretic blotting on nitrocellulose. First, immunoglobulins, complement components (C1q, C1s, C3) and [C-reactive protein] were found in IC [immune complexes]. Secondly, one mycobacterial antigen of 67 kD was directly identified in IC, while two other components (20 kD and 14.4 kD) of possible *Mycobacterium leprae* origin were also found in IC. This study suggests that lepromatous patients develop a good antibody response against some *M. leprae* antigens (33 kD and 12 kD), which are rapidly eliminated from circulation, while other *M. leprae* antigens (e.g., 67 kD) can persist in relative antigen excess, within circulating IC.—(*From* Trop. Dis. Bull.)

Lim, S. D., Woo, C. S., Youn, J. I., Kim, Y. W., Kim, D. I. and Fusaro, R. M. Leprosy XII. T-cell subsets in lepromatous leprosy. Int. J. Dermatol. **21** (1982) 458–464.

The authors quantitated T-rosette-forming cell (TRFC) and T-cell subsets (T_μ, T_γ) in the peripheral blood of 20 patients with lepromatous leprosy. The results obtained in their studies are as follows: 1) They reconfirmed the low levels of TRFC in patients with lepromatous type of leprosy; 2) T cell subsets, both T_μ (helper) and T_γ (suppressor) cells, showed lower levels in all patients with lepromatous leprosy than mean values of normal healthy controls; 3) The degree of decreased levels of T_μ cells (96%) was more severe than other parameters TRFC (70%) and T_γ cells (47%) in all patients with lepromatous leprosy; and 4) It may be concluded that the alteration of the T cell subset, T_μ cells, reflects a more fundamental abnormality than TRFC aberration in demonstrating the impairment of cell-mediated immunity in patients with lepromatous leprosy.—Authors' Abstract

Mathur, N. K., Mangal, H. N., Mathur, D., Bedwal, R. S. and Mathur, R. S. Langerhans cell and leprosy. Lepr. India **55** (1983) 22–28.

Langerhans cell (LC) population was counted in 44 patients of different types of leprosy and compared with 12 normal volunteers. A significant reduction in LC count was observed in cases of LL ($253.44 \pm 136.83/mm^2$) and BL ($349.36 \pm 121.67/mm^2$); whereas in TT ($854.60 \pm 332.01/mm^2$) and BT ($715.76 \pm 235.33/mm^2$) there was no significant difference as compared to normal ($927.43 \pm 103.87/mm^2$). Treatment had no influence on LC population in both the polar types of leprosy. The role of these immunocompetent dendritic cells in the pathogenesis of leprosy is discussed.—Authors' Summary

Miller, R. A., Dissanayake, S. and Buchanan, T. M. Development of an enzyme-linked immunosorbent assay using arabinomannan from *Mycobacterium smegmatis*: A potentially useful screening test for the diagnosis of incubating leprosy. Am. J. Trop. Med. Hyg. **32** (1983) 555–564.

A carbohydrate antigen composed predominantly of arabinomannan has been purified from *Mycobacterium smegmatis* and used in an enzyme-linked immunosorbent assay to detect anti-mycobacterial antibodies in human sera. Sera from 117 controls, 25 tuberculosis patients, 124 leprosy patients and 256 household contacts of leprosy patients were tested. When compared with the control group, 56% of tuberculosis patients, 27% of patients with tuberculoid leprosy, 77% of borderline leprosy cases, and 95% of patients with lepromatous leprosy had elevated titers. Nine percent of the household contact group had abnormally high levels of antibody. The relevance of these findings to the serodiagnosis of incubating leprosy and the management of household contacts of leprosy patients is discussed.—Authors' Abstract

Moura, N. C., Longo, I. M., Bernd, L. A. G. and Mendes, N. F. Quantitation of the soluble receptor of human T lymphocytes for sheep erythrocytes by electroimmunodiffusion in the serum of patients with cancer, uremia and leprosy. Experientia **39** (1983) 306–308.

Abnormally high serum levels of the soluble receptor of human T lymphocytes for sheep erythrocytes were found, by electroimmunodiffusion, in patients with carcinoma or other solid tumors, leukemia, lymphoma, uremia, and lepromatous leprosy.—Authors' Summary

Mustafa, A. S. and Godal, T. *In vitro* induction of human suppressor T cells by mycobacterial antigens. BCG activated $OKT4^+$ cells mediate suppression of antigen induced T cell proliferation. Clin. Exp. Immunol. **52** (1983) 29–37.

Peripheral blood mononuclear cells (PBMC), obtained from BCG vaccinated healthy donors, were induced to proliferation by BCG for five days *in vitro*. When re-exposed to BCG, they failed to proliferate. However, they partially retained the ability to respond to Con A and allogeneic cells. The addition of graded numbers of such cultured cells to fresh autologous

PBMC suppressed their proliferative response to BCG. These suppressor cells could also inhibit the proliferation of fresh cells to other mycobacterial antigens, both in particulate form, i.e., *Mycobacterium leprae,* or in soluble form, i.e., PPD and SPA30. However, these pre-cultured cells did not inhibit the response of fresh cells to non-specific mitogens, i.e., ConA and alloantigens. The inhibition of the response to non-mycobacterial soluble antigens, i.e., tetanus toxoid (TT) and diphtheria toxoid (DT), varied with little suppression in some individuals and stronger suppression in others. The suppression to BCG was found to be mediated by T cells. Subfractionation of T cells by monoclonal antibodies OKT4 and OKT8 allocated the suppressor cells to the $OKT4^+$ class of T cells. The suppression in the autologous system was quite strong, whereas it was much weaker in allogeneic systems.—Authors' Summary

Stach, J. L., Strobel, M., Fumoux, F. and Bach, J. F. Defect in the generation of cytotoxic T cells in lepromatous leprosy. Clin. Exp. Immunol. **48** (1982) 633–640.

Cytotoxic T cells are consistently produced in normal individuals after *in vitro* stimulation by a pool of mitomycin-treated normal lymphocytes. Patients suffering from lepromatous leprosy (LL), presenting with large amounts of *Mycobacterium leprae* and without a history of erythema nodosum leprosum (ENL), are unable to generate such cytotoxic T cells, while lepromatous patients with ENL which, in the present study [from Senegal] were all deprived of *M. leprae,* react normally.—(*From* Trop. Dis. Bull.)

van Eden, W., Elferink, D. and de Vries, R. R. P. An approach to study *in vitro* the expression of HLA-encoded genetic factors predisposing to tuberculoid leprosy. J. Immunogenet. **10** (1983) 107–114.

The existence of HLA-encoded genetic factors controlling susceptibility to tuberculoid leprosy in humans has been firmly established. Furthermore HLA-DR2 has been recognized as a marker for tuberculoid leprosy in India. At this moment, however, the gene products involved and the mechanism by which they confer susceptibility to tuberculoid leprosy remain only speculative. In an attempt to detect *in vitro* the expression of these HLA-encoded factors, we studied 12 tuberculoid leprosy patients and 22 healthy family members in a lymphocyte transformation test (LTT). All individuals were derived from multi-case Indian families, previously reported to show the presence of HLA-linked susceptibility genes. Although the responder status of the healthy contact siblings was shown to behave independently of whether they were HLA-identical with the patient—siblings or not, some evidence for *in vitro* expression of HLA-DR2 associated factors could be obtained. Nevertheless, it is concluded that the standard LTT seems not to be a test system of first choice to detect the *in vitro* expression of the genes under study.—Authors' Summary

Wadee, A. A., Mendelsohn, D. and Rabson, A. R. Characterization of a suppressor cell-activating factor (SCAF) released by adherent cells treated with *M. tuberculosis.* J. Immunol. **130** (1983) 2266–2270.

Peripheral blood adherent cells ingesting killed *Mycobacterium tuberculosis* release a suppressor cell-activating factor (SCAF) into their culture supernatants. When adherent cells ingested ^{125}I-labeled *M. tuberculosis,* radioactivity could be detected in the supernatant within 2 hr. When this supernatant was fractionated on a Sepharose 2B column, the fraction with suppressor cell-activating activity was also found to contain the majority of the radiolabel, which suggests that the macrophage processed bacteria (or bacterial product) constituted the major portion of the SCAF. This fraction also contained a high proportion of lipid, and the fraction with suppressor activity resided purely within the phospholipid fraction. By employing thin-layer chromatography, the phospholipids responsible were identified as phosphatidylethanolamine and phosphatidylinositol. These results indicate that when macrophages ingest mycobacteria, they release phosphatidylethanolamine and phosphatidylinositol of bacterial origin into their culture supernatants, which are responsible for activating suppressor T cells.—Authors' Summary

Wadee, A. A. and Rabson, A. R. Binding of phosphatidylethanolamine and phosphatidylinositol to OKT 8+ lymphocytes activates suppressor cell activity. J. Immunol. **130** (1983) 2271–2276.

The phospholipids phosphatidylethanolamine (PE) and phosphatidylinositol (PI) have been shown to activate a population of OKT 8-enriched lymphocytes to become activated suppressor cells that result in the suppression of lymphocyte blastogenesis to a variety of mitogens and antigens. This suppression is dose dependent, and maximal suppressor activity is obtained at concentrations of 125 μg/ml PE and 25 μg/ml PI. Activation of the suppressor cell population is not associated with an actual increase in the number of cells expressing the OKT 8 antigen, but the number of these cells expressing Dr antigens on their surface was increased. Both PE and PI bound to lymphocytes in a specific manner. Binding of radiolabeled PE could be inhibited by unlabeled PE but not by PI or phosphatidylserine (PS). Similarly, the binding of PI to lymphocytes was also found to be specific. Although radiolabeled PE bound to lymphocytes other than OKT 8^+ cells, and to other peripheral leukocytes, it bound to OKT 8^+ cells with a significantly greater affinity than it did to the other cell types. The Kd for PE was 1×10^2 nM and for PI was 1×10^3 nM, and receptor cell densities for these two phospholipids were estimated at 1×10^{-8} nM and 3×10^{-9} nM, respectively. The receptors for these two phospholipids were trypsin and heat sensitive, and the receptor sites could be regenerated after a 24-hr incubation after trypsinization.—Authors' Summary

Microbiology

Andersen, O., Jantzen, E., Closs, O., Harboe, M., Saxegaard, F. and Fodstad, F. Fatty acid and polar lipid analysis as tools in the identification of *Mycobacterium leprae* and some related slow-growing mycobacterial species. Ann. Microbiol. (Paris) **133B** (1982) 29–37.

Some species of slow-growing mycobacteria, including *Mycobacterium leprae* (1 strain), *M. lepraemurium* (2 strains), *M. paratuberculosis* (12 strains) and a group of 12 *M. avium*-like strains (isolates from wild animals) were examined by gas chromatography (GC) for cellular fatty acids and by thin-layer chromatography (TLC) for polar lipids. All the GC patterns, including that of *M. leprae,* contained high levels of tuberculostearic-, stearic-, octadecanoic- and palmitic acid. Tetradecanoic-, pentadecanoic-, hexadecanoic- and heptodecanoic acid were also generally present but in lower concentrations. In addition to these acids shared by all strains, each bacterial species or group was found to exhibit compounds which were not detected (or detected in considerably lower quantities) in the other taxa examined. Thus each bacterial species or group could be distinguished by their GC profiles. The corresponding TLC patterns were also rather complex. A total of 39 different spots were distinguished. A few of these were shared by all strains; some were characteristic of certain species or groups, whereas others were strain-specific. Both *M. leprae* and *M. lepraemurium* shared several features with the other strains but could be distinguished from each other and from the others by their patterns of slow-moving (polar) spots. The 12 *M. avium*-like strains were divided into two main groups, one with only a few slow-moving spots (rough strains), and one with several slow-moving spots (smooth strains) which included the *M. avium* reference strains.—Authors' Summary

David, H. L., Rastogi, N., Frehel, C. and Gheorghiu, M. Reduction of potassium tellurite and ATP content in *Mycobacterium leprae.* Ann. Microbiol. (Paris) **133B** (1982) 129–139.

The purpose of this investigation was to examine those properties of *Mycobacterium leprae* which might be useful in estimating the heterogeneity of the bacterial populations harvested from the tissues of experimentally infected armadillos. The following

technical procedures were applied: fluorescent microscopy on smears stained by an auramine o-ethidium bromide dual procedure, fine structure observation of ultrathin sections, reduction of potassium tellurite as observed under the electron microscope, and ATP content of the bacteria. The quantitative data from these procedures were compared to the Morphological Index, and it was shown that the results did not correlate. However, the study of tellurite reduction was interesting because this technique may prove useful in evaluating the contamination of *M. leprae* preparations by host tissues. More significant was the fact that *M. leprae* reduced tellurite. Even though the reduction was at a very low efficiency under the conditions described, it would be possible to use this technique to investigate the effects of several substrates on the respiratory activity of the leprosy bacilli.—Authors' Summary

Dhople, A. M. Taxonomic studies on *Mycobacterium leprae.* Lepr. India **55** (1983) 39–44.

Based on biochemical properties, *M. leprae* harvested from armadillos has been shown to resemble more closely with *M. vaccae* than with other selected cultivable mycobacteria.—Author's Summary

Draper, P., Dobson, G., Minnikin, D. E. and Minnikin, S. M. The mycolic acids of *Mycobacterium leprae* harvested from experimentally infected nine-banded armadillos. Ann. Microbiol. (Paris) **133B** (1982) 39–47.

Mycolic acid methyl esters were prepared from defatted cells of armadillo-derived *Mycobacterium leprae* and analyzed by thin-layer and high-performance liquid chromatography, proton magnetic resonance spectrometry and mass spectroscopy. The first type of mycolic acid characterized was an "α-mycolate" having two *cis*-cyclopropane rings, a 78-carbon main component and an overall size-range of 72 to 83 carbons. Ketomycolates, with an 83-carbon main component, were the only other type of mycolate isolated; the major 79- to 87-carbon series of ketomycolates apparently contained a single *trans*-cyclopropane and the minor 80- to 86-carbon series had a *cis*-cyclopropane function.—Authors' Summary

Draper, P., Payne, S. N., Dobson, G. and Minnikin, D. E. Isolation of a characteristic phthiocerol dimycocerosate from *Mycobacterium leprae.* J. Gen. Microbiol. **129** (1983) 859–863.

A characteristic mycobacterial wax, phthiocerol dimycocerosate, has been isolated from livers of armadillos experimentally infected with *Mycobacterium leprae.* The structure of this wax is generally similar to that produced by *M. tuberculosis,* but the homologous phthiocerol and the mycocerosic acid components from *M. leprae* are significantly different from those of *M. tuberculosis.*—Authors' Summary

Mittal, A., Sathish, M., Seshadri, P. S. and Nath, I. Rapid, radiolabeled-microculture method that uses macrophages for *in vitro* evaluation of *Mycobacterium leprae* viability and drug susceptibility. J. Clin. Microbiol. **17** (1983) 704–707.

This paper describes a microculture rapid assay using radiolabeling and mouse macrophages to determine the viability and the drug susceptibility or resistance of *Mycobacterium leprae.* Comparison of *M. leprae*-resident macrophage cultures maintained in 96-well flat-bottomed plates showed results for viability and susceptibility or resistance to dapsone that were similar to results for concurrent cultures in Leighton tubes with greater numbers of bacilli and macrophages.—Authors' Summary

Portaels, F. and Pattyn, S. R. Growth of mycobacteria in relation to the pH of the medium. Ann. Microbiol. (Paris) **133B** (1982) 147.

The influence of pH on the growth of mycobacteria on Löwenstein and Dubos OAA media was studied on 56 strains belonging to 10 species of rapidly growing and 16 species of slowly growing mycobacteria. pH gradients were obtained using McIlvaine's citrate and Sörensen's citrate buffer.

The sensitivity of mycobacteria for different pH values of the medium is independent of the culture medium and the buffer used.

Minimal, maximal and optimal pH values are specific for each species, all strains of the same species producing identical results.

Some slowly growing mycobacteria develop within a very narrow optimal pH range, e.g., *Mycobacterium lepraemurium* (*Mlm*): between pH 5.8 and 6.1. Other species develop over a wide optimal pH range, e.g., *M. nonchromogenicum*: from 5 to >7.4. For slow growers in general, with the exception of *Mlm*, optimal pH is between 5.8 and 6.5.

In general, rapidly growing mycobacteria develop over a wider pH range of more than 2 units. All strains, with the exception of *M. chelonei*, develop optimally between pH 7 and 7.4. For *M. chelonei* the optimal pH—as for slower growing species—is between 5.4 and 6.5.

Taxonomically related species such as *M. tuberculosis* and *M. bovis*, *M. kansasii* and *M. gastri*, *M. microti* and BCG, *M. vaccae* and *M. parafortuitum* are inhibited or stimulated at identical pH values. This specificity constitutes a supplementary taxonomic character.

At certain pH values some species, otherwise sometimes difficult to separate, may be differentiated, e.g., *M. avium*, which does not grow at pH 4.6, as compared to *M. scrofulaceum*, which does grow at pH 4.6.

For routine diagnostic purposes, Löwenstein-Jensen medium at pH 7 is not optimal; Ogawa medium, with a pH of 6, is more appropriate.—Authors' Abstract

Portaels, F. and Pattyn, S. R. Taxonomy of *Mycobacterium leprae* and *M. lepraemurium*. Ann. Microbiol. (Paris) **133B** (1982) 99–108.

The characteristics of *Mycobacterium leprae* and *M. lepraemurium* are compared and it is concluded that they are quite separate species.

The Ziehl-Neelsen stain shows their morphology to be distinctive but this is not a suitable taxonomic character and the ultrastructure of the cell wall resembles that of other mycobacteria. Both species contain peptidoglycans in the cell wall, but, whereas in other mycobacteria this is rich in alanine, in *M. leprae* glycine predominates. Mycolic acids are also present but *M. leprae* lacks dicarboxymycolates which are present in most other mycobacteria. The species also lacks or has very low levels of tuberculostearic acid.

Studies of antigenic structure have revealed that *M. lepraemurium* is closely related to *M. avium* and that *M. leprae* has specific antigens. With the use of *M. lepraemurium* grown *in vitro* it is now evident that much of the work on metabolic activity is suspect, presumably because organisms grown *in vivo* metabolize substances in a different manner. Metabolic tests for *M. leprae* appear to be variable and work is needed to standardize the cell suspension, for example, in the dihydroxyphenylalanine (DOPA) oxidase test.

Dapsone has previously been thought to be specific for *M. leprae* but mycobacteria recently isolated from the environment are also sensitive to this drug. *M. lepraemurium* has a distinctive drug-sensitivity pattern. The growth of *M. leprae* in the foot pad of the mouse is distinctive and allows it to be distinguished from *M. lepraemurium* and other mycobacteria that can to some extent infect the mouse and from the environmental strains that are sensitive to dapsone.

Tables present the *in vitro* characters of *M. lepraemurium* compared with *M. avium*, *M. paratuberculosis* and *M. haemophilum*, showing that they are all different, and characteristics useful for identifying *M. leprae*.—P. A. Jenkins (*From* Trop. Dis. Bull.)

Rastogi, N., Frehel, C., Ryter, A. and David, H. L. Comparative ultrastructure of *Mycobacterium leprae* and *M. avium* grown in experimental hosts. Ann. Microbiol. (Paris) **133B** (1982) 109–128.

To ascertain whether the ultrastructural features characteristic of *Mycobacterium leprae* were partially determined by the growth of these bacteria in animal host tissues, the ultrastructure of host-grown *M. leprae* was compared to that of host-grown *M. avium*. The bacteria were studied in the livers of experimental hosts, which were armadillo and rabbit for *M. leprae* and *M. avium*, respectively. The ultrastructural examinations were performed on tissue sections as well as on isolated bacteria. In both cases, the bacteria were examined after ura-

nyl acetate and lead citrate staining, silver proteinate coloration, acidic phosphotungstic staining, and ruthenium red coloration. In the armadillo liver, essentially three types of *M. leprae* were observed: bacilli containing all structures of a procaryotic cell (a well-defined cell wall and cytoplasmic membrane around a granulated cytoplasm with visible mesosomes, ribosomes and nucleoplasm); bacilli similar to the above but with a homogenous cytoplasm; and bacilli in which no regular ultrastructure could be observed and which very rarely contained vacuoles. The relative frequency of the three types of cells was, respectively, 5%–10%, 80% and less than 10%. On the other hand, in the rabbit liver, only intact *M. avium* bacilli with a well-developed ultrastructure were observed. In any case, both host-grown *M. leprae* and *M. avium* contained an outer electron-transparent zone (ETZ), had the same location of an α-1-2-glycol bond containing polysaccharides (in the cytoplasmic membrane and in mesosomes), had the same organization of the peptidoglycan layer (though thicker in *M. avium*) and, finally, contained a deposit of polysaccharides at the outer surface of their respective ETZ. However, contrary to the first subculture of the host-grown *M. avium* cells—where this polysaccharide outer layer (POL) was thicker and uniformly visible—the POL was not always visible and could not be detected regularly around all host-grown *M. leprae* and *M. avium* cells. The host-grown *M. avium* and *M. leprae* were rich in huge cytoplasmic inclusions of polysaccharides which were rich in α-1-2-glycol bonds. These inclusions were absent in the case of laboratory-maintained *M. avium* cells. As these inclusions were even lost from the host-grown *M. avium* after 1–2 subcultures in laboratory culture conditions, they are probably specific for a host-parasite relationship. Finally, the host-grown *M. avium* were rich in paracrystalline inclusions, which were completely absent after one or more subcultures of the same bacteria in the laboratory. Similar structures had been reported earlier for *M. leprae*.—Authors' Summary

Silva, M. T. and Macedo, P. M. Ultrastructure of *Mycobacterium leprae* and other acid-fast bacteria as influenced by fixation conditions. Ann. Microbiol. (Paris) **133B** (1982) 59–73.

A procedure using aldehydes, OsO_4, Ca^{++} and uranyl acetate was selected for study of the fixation of *Mycobacterium leprae* in skin biopsies of leprosy patients. The ultrastructural pattern of recognized normal *M. leprae* cells fixed by the above procedure was characterized, and was found to be similar to that of other acid-fast bacteria fixed by the same procedure, except for the geometry of the membrane profile. Under such fixation conditions, this profile is always asymmetric in *in vitro*-cultured normal *Nocardia asteroides, M. aurum* and *M. tuberculosis*; whereas in skin biopsies, no *M. leprae* cells with asymmetric membranes have been found so far. The implications of this observation for the interpretation of the ultrastructure of damaged *M. leprae* cells found in skin biopsies are discussed.—Authors' Summary

Sula, L. The microcolonial texture and demonstration of a phage-like lysis in the "Douglas" strain of *Mycobacterium lepraemurium*. Ann. Microbiol. (Paris) **133B** (1982) 153.

A technique using thin sections of Ogawa egg yolk culture medium inoculated with *Mycobacterium lepraemurium* was described as being capable of demonstrating microscopical growth of this strain, which did not show up on macroscopical examination. A peculiar structure of this growth—characterized by many lytic spots of different size—was encountered, indicating the possible presence of a temperate phage.—Author's Abstract

Wieten, G., Haverkamp, J., Berwald, L. G., Groothuis, D. G. and Draper, P. Pyrolysis mass spectrometry: its applicability to mycobacteriology, including *Mycobacterium leprae*. Ann. Microbiol. (Paris) **133B** (1982) 15–27.

Pyrolysis mass spectrometry is a technique for the analysis of complex organic substances including bacterial fractions and intact bacteria. It has already been used to compare various mycobacteria and to assign unknown strains to defined groups. This paper describes its use in the study of the

heterogeneity of *Mycobacterium africanum,* of different batches of *M. leprae* and in a comparison of *M. leprae* and mycobacteria thought to be closely related.

Details of the technique and the computerized processing of the data are beyond the scope of the abstract. However, each strain produces a spectrum composed of discrete mass/charge ratios with varying intensities and these can be related to, for example, particular proteins or carbohydrates, and constitute a profile of the strain. Relationships between strains are portrayed in non-linear maps which are two-dimensional representations of a multi-dimensional dissimilarity matrix.

The ten strains of *M. africanum* formed a continuous extended cluster, but clearly belonged to the same species. The largest variations corresponded to the axis of geographical variation with strains from Rwanda at one end and from Dakar at the other.

Of the 6 different batches of *M. leprae* prepared from armadillo livers, 4 formed a distinct cluster, but the other 2 were different. The differences may have been due to the presence of varying amounts of contaminating material from the armadillo liver or from substances used in the extraction procedure, such as poly(ethyleneglycol). It is possible that pyrolysis mass spectrometry will be of use in checking the purity of *M. leprae* preparations since it can detect very small amounts of contaminating substances.

The comparison of *M. leprae* with related mycobacteria showed it to be distinct from such organisms as *M. scrofulaceum, M. vaccae, M. nonchromogenicum,* the Skinsnes strain and a provisional new species *M. lufu.* However, it is acknowledged that the method of preparing *M. leprae* is so different from the way other mycobacteria are grown that this alone could give rise to the differences observed.

[This technique promises to be of great value in broadening our understanding of the mycobacteria. However, it requires expensive equipment and considerable expertise in its performance and is, therefore, likely to remain a research tool for a long time.]—P. A. Jenkins (*From* Trop. Dis. Bull.)

Experimental Infections

Adu, H. O., Turk, J. L. and Curtis, J. The histopathology of tissues in "resistant" and "susceptible" strains of mice infected with a moderate dose of *Mycobacterium lepraemurium.* J. Pathol. **139** (1983) 275–290.

A systematic study by light and electron microscopy of tissues from BALB/c and C57BL/6 mice infected subcutaneously with 10^7 *Mycobacterium lepraemurium* organisms was carried out at various times throughout the infection. The relatively resistant C57BL/6 mice had an earlier inflammatory response at the site of the infection than did the susceptible BALB/c mice. The infiltration in the former strain contained fibroblast-like cells and epithelioid cells early on in the infection. Few lymphocytes were observed in both strains throughout the infection. The spread of acid-fast bacilli was slower in the resistant strain (C57BL/6). The findings indicated that the rate of cellular infiltration at the infection site and the nature of the cells in the infiltration may determine the outcome of this infection.—Authors' Summary

Bhat, K. R. and Vaidya, M. C. Vascular changes in nerves in experimental leprosy—an ultrastructural study. Lepr. India **55** (1983) 60–63.

Ultrastructural observations made on the blood vessels of nerves in CBA/J mice with experimentally produced *M. leprae* infection revealed cytoplasmic filamentous structures along with large-size vacuoles in the endothelial cells and reduplication of peripericytal basement membranes in the 6–9 months of infection. In animals with late infection of 9–15 months' duration, the endothelial cells presented a foamy appearance caused by an increase in the number of vacuoles. These findings are suggestive of degenerative changes in the endothelial cells probably due to circulation of a noxious substance in the blood stream.—Authors' Summary

Ganguly, N. K., Kumar, B., Kaur, S., Vaishnavi, C. and Chakravarti, R. N. Lymphocyte subpopulations in mice infected with *Mycobacterium leprae*. Lepr. India **55** (1983) 29–38.

T and B cells were quantitated from the spleen of *M. leprae*-infected mice and correlated with bacillary count in the foot pad. Lymphocyte transformation with PHA and *M. leprae* (armadillo) antigens was also studied during different months of infection. T cell counts dropped gradually but significantly throughout the course of infection. B cells had a concomitant rise up to six months and then registered a fall as compared to the initial control figures. Transformation of lymphocytes with PHA fell significantly after the fourth month until the end of the experiment; whereas the stimulation index for armadillo antigen rose gradually from the third month onwards to reach a peak at the sixth month and then fell until the end of the experiment with increasing bacterial population. B cell counts showed little change.—Authors' Summary

Lagrange, P. H., Hurtrel, B., Ravisse, P. and Grosset, J. A single subcutaneous inoculation of 10^7 armadillo-derived irradiated *Mycobacterium leprae* evokes different immunological behavior in C57BL/6 and C3H mice. Ann. Microbiol. (Paris) **133B** (1982) 167–168.

Following subcutaneous injection in the foot pad with 6.7×10^3 living *Mycobacterium leprae,* it was observed that locally, at the injection site, more microorganisms were destroyed after the sixth month in C57BL/6 mice than in C3H/HeN mice. The specific and non-specific immune responses after inoculations of armadillo-derived, irradiated killed *M. leprae* (IML) was then investigated in these two strains of mice and also in Biozzi's HL and LL mice. One single injection of 1×10^7 IML into the hind foot pad of C57BL/6 and Biozzi's HL mice was able to elicit a state of cell-mediated immunity (CMI) to *M. leprae* antigens or to crossreacting mycobacterial antigens, such as BCG antigens. The selected parameters of CMI were as follows:

a) Development of an immune granuloma at the injection site and in the draining lymph node which corresponds to the accumulation of macrophages and lymphoid cells.

b) Development of a systemic delayed-type hypersensitivity (DTH) to both living BCG and IML.

c) Specific acquired protection against infection with *M. bovis* strain BCG.

d) Immunopotentiation of the DTH response to an unrelated antigen, such as sheep red blood cells.

In low responder strains (C3H/HeN and low-antibody producer mice selected by Biozzi) no such parameters developed at all after immunization.—Adapted from Abstract

Saito, N. and Hirooka, Y. Evidence for generation of suppressor T cells in *Mycobacterium lepraemurium*-infected CBA/J mice, a mouse strain highly susceptible to the infection. Microbiol. Immunol. **27** (1983) 75–85.

Susceptibility to *Mycobacterium lepraemurium* (Mlm) infection markedly differed between two mouse strains, CBA/J and C57BL/6. CBA/J mice showed high susceptibility to Mlm infection and developed either very weak or no delayed-type hypersensitivity (DTH) to Mlm antigen after the injection of Mlm. In contrast, C57BL/6 mice, which were resistant to Mlm infection, showed significant DTH reaction to Mlm antigen after the injection.

Treatment of CBA/J mice with cyclophosphamide (Cy) conferred significant resistance to Mlm infection on the CBA/J mice, and the treated mice developed a strong anti-Mlm DTH response after the Mlm injection. When spleen cells from Mlm-infected CBA/J mice were transferred to Cy-treated and Mlm-infected syngeneic mice, the anti-Mlm DTH reaction of the recipient mice was suppressed. Treatment of the spleen cells to be transferred with anti-Thy-1.2 antibody or anti-I-J^k antiserum plus complement abrogated the suppressive activity. Thus, it is suggested that the high susceptibility of CBA/J mice to Mlm infection is due to the generation of Cy-sensitive, I-J^k-positive suppressor T cells after infection with Mlm.—Authors' Abstract

Epidemiology and Prevention

Agüero, H., Arpini, R., Casim, G., Recaret, M., Boja, M. D., Chapo, R. and Zaffora, S. Método de recolección de datos para registro estadístico de pacientes de lepra. [The method of retrieval of data for statistical registration of leprosy patients.] Rev. Argent. Dermatol. **64** (1983) 49–51. (in Spanish)

A file registering leprosy patient data is suggested, to be adopted as a pattern to compare statistical data in the various services. It contains multiple variables, and it is advisable not to employ all of them together in different research projects. The data collected are the most important within modern leprology.—Authors' Summary

Arpini, R. H., Casim, G., Chapo, R., Zaffora, S., Recarte, M., Boja, M. D., Ekdesman, R. and Escalona, M. Registro de pacientes del Servicio de Dermatoleprología del Policlínico Intendente Carrasco de Rosario. [Registry of patients of the Service of Dermatoleprology of the Policlinico Intendente Carrasco de Rosario.] Rev. Argent. Dermatol. **64** (1983) 53–65. (in Spanish)

This is a descriptive paper on the register of patients from the Policlinic "Intendant Carrasco," from its beginning until 1981. It has been a study of 2819 patients, 1546 men and 1273 women, from that register. Up to that time it is possible to know that 657 patients, 350 men and 370 women attend this hospital. They constitute 350 lepromatous patients, 95 tuberculoid ones, 160 patients of indeterminate leprosy, 46 patients of borderline type and 6 patients without a very defined clinical form.—Authors' Summary

Arpini, R., Casim, G., Chapo, R., Zaffora, S., Recarte, M. and Escalona, M. Lepra infantil en el registro de pacientes del Servicio de Leprología del Policlínico Intendente Carrasco de Rosario. [Infantile leprosy in the registry of patients of the Service of Leprology of the Policlinico Intendente Carrasco de Rosario.] Rev. Argent. Dermatol. **64** (1983) 67–72. (in Spanish)

A statistical study of leprosy patients registered at the Carrasco Hospital (Rosario, Argentine Republic) was performed. A incidence of 5.42% of leprosy in infants (153 cases) was noted.

The following features were computed among these patients: age, sex, nationality, origin, clinical form, source of infection, age at appearance of the first symptoms, delay between the discovery of the first symptoms and the first medical consultation, baciloscopy, immunological findings.—Authors' Summary

Serjeantson, S. W. HLA and susceptibility to leprosy. Immunol. Rev. **70** (1983) 89–112.

This review examines the evidence for involvement of MHC-associated factors in host immune response to *Mycobacterium leprae,* by collating HLA studies of sporadic and familial leprosy and discussing possible HLA-related immunological mechanisms in determining host response.

Formal linkage analysis of 109 multiple-case families with data available for HLA haplotype segregation showed that under a three-allele recessive model for susceptibility to leprosy, linkage was observed between the HLA complex and a leprosy susceptibility locus at a recombination fraction of 20%. The significance of the linkage relationship was confined to families with at least two tuberculoid leprosy offspring and neither parent affected. When one parent was affected, with leprosy of any clinical type, lod scores could neither implicate nor exclude linkage between HLA and leprosy susceptibility and this apparent paradox can be explained by the presence of an additional, non-HLA linked susceptibility locus for leprosy.—Author's Summary

Tuberculosis and leprosy control; WHO consultation. WHO Wkly. Epidemiol. Record **58** (1983) 109–112. (in English and French)

Recent information indicates that the epidemiological behavior of tuberculosis in some tropical areas may be substantially different from the well-known pattern of the

disease in developed countries. Wide gaps in knowledge have emerged, calling for a revision of present research priorities. The epidemiology of leprosy is even less well understood than that of tuberculosis, a major problem being the absence of an appropriate method to measure the prevalence and risk of infection.

A consultative group of epidemiologists met in Geneva, 8–12 November 1982, to identify the main problems which are of immediate importance for tuberculosis and leprosy control, and to indicate areas for research.

In leprosy, in general, studies which have a greater relevance to control should receive priority. In broad terms the studies could be grouped into: a) research which can be undertaken with available knowledge and methods, and b) research which requires new tools.

The following studies are recommended under a):

Prevalence studies, including sample surveys, to measure the problem and its various dimensions including distribution by type of disease, age, sex, contact status, etc. There is a need to standardize criteria for diagnosis of disease, classification, and activity. The uneven distribution of leprosy warrants appropriate designs for sample surveys.

Incidence studies in selected areas to identify, where possible, risk factors, vulnerable groups, and the trend of the disease through changes in its distribution by form, contact status, age, sex, etc. Such studies would be valuable for future vaccine trials.

Pathogenesis of leprosy in different regions, particularly the evolution of multibacillary and other progressive forms, and factors contributing to the downgrading of other forms of leprosy to multibacillary leprosy.

Studies on the impact of multidrug therapy, through prevalence and incidence studies over a period of time, particularly in younger age groups. When tools for identifying subclinical infection become available, the incidence of infection should also be studied.

Studies on transmission, particularly in relation to attack rates among contacts under different conditions and factors that influence transmission among contacts.

Studies on the interaction between leprosy and environmental mycobacteria.

Epidemiological studies on drug resistance, particularly in relation to the infectivity of drug-resistant cases.

Re-analysis of all available data from the BCG trials, including the small scale studies on selected groups such as contacts, to see whether there is a common pattern of protection against leprosy.

Case-control studies may help to assess the value of BCG in other areas.

It is likely that reliable immunological tools may become available in the near future, both through serological tests and skin tests. It will be necessary to prepare an inventory of studies that should be carried out when such tools become available.

The studies recommended under b) may include:

Studies to correlate infection with disease under different conditions.

Studies to identify groups at high risk of developing lepromatous leprosy.

Studies on the infectivity of different types of leprosy under different conditions.

Studies to test endogenous reactivation and the reinfection hypothesis in leprosy.

There are similarities, differences and interactions between tuberculosis and leprosy which are imperfectly understood. Two main areas should be explored:

The epidemiological interactions between tuberculosis and leprosy infection and disease, and the immunological role of other mycobacterial infections, particularly in relation to the information being collected in the Chingleput trial.

Health services research on the possibility of combining control programs for tuberculosis and leprosy, and incorporating the control of these two diseases into the primary health care system.

—(*From* the Report)

Rehabilitation

Jones, R. O. Ulceration in the neurotrophic foot of Hansen's disease. J. Am. Podiatry Assoc. **72** (1982) 299–303.

Untreated, Hansen's disease can cause scarring, swelling, and disfigurement of the body, crippling of the hands and feet, loss of feeling in affected areas, and blindness. Even though Hansen's disease has many complications, in this Korean experience plantar ulceration has proven to be the most common and leads to much disability. Ulceration in leprosy neurotrophic feet, detected early, is preventable.—Author's Summary

Pring, D. J. and Casabianca, N. "Dorsal incision"—the treatment of complicated forefoot plantar ulcers in the anaesthetic foot. Lepr. India **55** (1983) 49–59.

The in-patient treatment of 107 complicated forefoot plantar ulcers occurring in the anesthetic foot by the operation of dorsal incision is presented. The operative technique of dorsal incision and subsequent wound dressing is described. Sixty-nine ulcers healed prior to discharge and all but three ulcers healed in an average of 27.2 days. In-patient stay averaged 31.4 days. The complications of the procedure are presented. Emphasis is placed on health education of the patient regarding care of the anesthetic foot. Further follow up is suggested to see if dorsal incision reduces the recurrence of plantar ulceration.—Authors' Summary

Other Mycobacterial Diseases and Related Entities

Edwards, M. L., Goodrich, J. M., Muller, D., Pollack, A., Ziegler, J. E. and Smith, D. W. Infection with *Mycobacterium avium-intracellulare* and the protective effects of bacille Calmette-Guérin. J. Infect. Dis. **145** (1982) 733–741.

The efficacy of BCG vaccination trials against tuberculosis has ranged from 0%–80%, depending on the geographical location. The trial in South India showed negligible protective effect of BCG vaccination. Two of several explanations for this failure are explored experimentally in this paper. The first hypothesis tested is that atypical mycobacteria (known to be common in the trial area) themselves conferred a protective effect against tuberculosis which masked any protection afforded by the BCG. The second hypothesis is that these atypical mycobacteria somehow interfered with the protective effect of the BCG.

To test these hypotheses the authors injected groups of guinea pigs twice intradermally with 50 colony-forming units (cfu) of BCG (a batch used in the trial), 10^6 cfu mycobacteria of the *Mycobacterium avium-intracellulare* complex (MAI; isolated from sputum of patients in the trial area) or placebo so that all possible combinations of treatment had been given. There was a six-week interval between these injections. Six weeks after the second injection, the guinea pigs were challenged by the respiratory route with one of three strains of *M. tuberculosis.* These strains were H37Rv, and 2 strains isolated from patients in the trial area—1 with low (SI LV) and 1 with high (SI HV) virulence for guinea pigs. Tissues of representative animals were examined four or seven weeks later.

The results are presented in detail and deserve thorough reading by all interested in this topic. In summary, it was found that MAI was generally as effective as BCG in protecting the guinea pigs against challenge with H37Rv. The same was true against challenge with SI LV although it was thought that a longer interval between sensitization to MAI and challenge with pathogenic tubercle bacilli might be required as compared with the interval between BCG vaccination

and challenge. MAI was less effective than BCG in protection against SI HV. There was no evidence to suggest that prior sensitization with MAI interfered with the protective effect of BCG. The authors conclude "our experiment provides no basis for the rejection of the hypothesis that sensitization with atypical mycobacteria confers a protective effect comparable with that given by BCG vaccination".—C. A. Brown (*From* Trop. Dis. Bull.)