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CURRENT LITERATURE

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

General and Historical

Banerjee, A. Dr. Robert Greenhill Cochrane CGM MD FRCP DTM&H: leprogist par excellence. J. Med. Biogr. **4** (1996) 137–140.

Dr Robert Cochrane devoted his entire working life to the study and control of leprosy. Most of his working life was spent in India, with interludes in Britain and east Africa. He initiated epidemiological surveys of leprosy, was instrumental in the introduction of sulfones for the definitive therapy of the disease, and contributed significantly to the development of rehabilitation programs for sufferers from the disease. He campaigned actively for altering social attitudes to leprosy and latterly was in favor of replacing the often pejorative term with that of Hansen's disease. A devout Christian, he believed strongly in setting an example for others as an important means of introducing them to Christianity. At the same time, he was not a taciturn individual and had a well-developed sense of humor.—Author's Abstract

Ishii, N. Clinical leprosy in Japan. Nihon Hansenbyo Gakkai Zasshi **70** (2001) 145–149. (in Japanese)

The Leprosy Prevention Law was abolished at the end of March 1996. Since medical insurance for leprosy started in April 1996, dermatologists in clinics have to take care of leprosy patients. However, dermatologists have not learned enough about leprosy, and only a few of them are familiar with it. Japanese patients newly-diagnosed with leprosy in Japan have decreased, and patients who come from foreign countries to work in Japan have become more important in leprosy control. Therefore, it is important to educate dermatologists about leprosy. Recently, diagnostic guides including information about network systems have become available in book stores. It is possible to obtain all kinds of information about leprosy from the network systems.—Author's Abstract

Marlowe, S. N. and Lockwood, D. N. Update on leprosy. Hosp. Med. **62** (2001) 471–476.

Leprosy, a result of infection by *Mycobacterium leprae,* is a leading cause of peripheral neuropathy. The World Health Organization aimed to eliminate leprosy as a public health problem by 2000, but this has not been attained. Patients with leprosy continue to be present in the UK. The diagnosis of leprosy is frequently not considered, with resultant pathological and psychological problems for patients.— Authors' Abstract

Navon, L. Beggars, metaphors, and stigma: a missing link in the social history of leprosy. Soc. Hist. Med. **11** (1998) 89–105.

Students of leprosy stigma are at odds over its sources, intensity, and current persistence. On the basis of a study of leprosy in Thailand that combined an archival survey with anthropological fieldwork, the present article offers a different thesis on these issues from those that have been proposed thus far. The thesis suggests that prior to the discovery of a cure for the disease, its sufferers encountered ambivalent, rather than severely, stigmatizing reactions. Yet the public's selective exposure, mainly to beggars with the disease, paved the way for the perception of leprosy as the epitome of

stigmatization and to its transformation into a metaphor for degradation. Progress in the medical treatment of the disease significantly improved patients' social acceptance, but also allowed them to keep their illness a secret. Their consequent disappearance from the public eye turned the figurative use of leprosy in the spoken language into the main source of shaping its image. This development contributed to the irrefutability and perpetuation of the negative image, and even to its intensification to the extent of utter divorce from concrete reality. After expounding this thesis, the paper discusses its potential contribution to resolving the disputes over the roots, severity, and persistence of the leprosy stigma on the international level.—Author's Abstract

Parascandola, J. An exile in my own country: the confinement of leprosy patients at the United States National Leprosarium. Med. Secoli **10** (1998) 111–125.

Leprosy is a disease which has long been stigmatized and persons afflicted with it have frequently been segregated from the rest of society. This paper focuses on the evolution of policies concerning the confinement of patients at the national leprosarium operated by the United States Public Health Service (PHS) at Carville, Louisiana. After a brief review of the origins of the Louisiana Leper Home, which eventually became the national leprosarium, the paper traces changing attitudes and policies at Carville from 1921, when the PHS took control of the facility, to the 1950s.—Author's Abstract

Sabater, P. G. Discourse on the social illness: leprosy in the Viceroyalty of Nueva Granada in the transition from the 17th to the 18th century (Spanish). Dynamis **19** (1999) 401–428.

The significance of leprosy in the Viceroyalty of Nueva Granada in the transition from the 17th to the 18th century is analyzed. In addition, we analyze treatments recommended by physicians in the viceroyalty, which were closely related with the etiology and pathogenesis which all doctors attributed to Saint Lazarus' disease. The diversity of opinions led to different therapeutic measures, not only with regard to alleviating the patient's symptoms, but also with a view to preventing its spread to the rest of the population. As a guiding theme we use the theories defended by the most representative physicians in the viceroyalty, and the views of patients themselves and the society they lived in.—Author's Abstract

Sanchez, G. R. The battle against leprosy in Spain in the first half of the XX century. Asclepio **46** (1994) 79–91.

The present work deals with the measures taken in Spain during the first half of the XX century to fight leprosy. The main policy to curb this infectious and contagious disease consisted first in isolating the patients; nevertheless, as knowledge concerning the disease grew considerably, its preventive measures started to evolve during the period. Another point of decisive influence regarding the fight against leprosy was the intervention of the health authorities in the different historical moments and the social response to this evil.—Author's Abstract

Suzuki, N. Manase Dosan (the Elder) and leprosy. Nippon Ishigaku Zasshi **41** (1995) 349–368.

The aim of this paper is to describe the main features of Manase Dosan's (1507–1594) study and the treatment of leprosy. Contrary to general medical opinion in the Middle Ages that leprosy was the result of divine retribution, Dosan viewed leprosy as simply another disease and treated it accordingly from a medical perspective. Furthermore, the commonly-held belief, from the latter half of the 17th century onward, among Early Modern era doctors and also the general populace that leprosy was a hereditary disease, was not considered by Dosan. The foregoing two points can be explained by his rational approach to medicine, plus the fact that leprosy at the time was widely prevalent among all areas of society, not just restricted to particular house-

holds. It is, thus, fair to say that Dosan's medical philosophy reflected the state of Japanese society during the transition from medieval to the Early Modern period. It should be noted, however, that Dosan's view that leprosy was caused by meat-eating and overindulgence in sex gave rise to a new, negative image of the disease, and in so doing tied in with the Early Modern era prejudices against "lust", "intemperance" and "laziness".—Author's Abstract

World Health Organization. Leprosy. Weekly Epid. Rec. **76** (2001) 173–179.

A review on leprosy as one of the leading causes of permanent physical disability in the world is presented. Early detection and cure using multidrug therapy (MDT) as the key elements of the strategy to eliminate leprosy as a public health problem are emphasized. The latest available information on prevalence and detection of leprosy by countries with more than 100 registered cases, grouped by WHO region, are tabulated. Elements of the intensified strategy for the remaining endemic countries are discussed. It is concluded that a strategy of early detection and treatment will not only stop the transmission of leprosy, but also prevent disabilities and thereby halt the downward spiral toward social exclusion and destitution.—Trop. Dis. Bull.

Chemotherapy

Ahmad, S. I. Control of skin infections by a combined action of ultraviolet A (from sun or UVA lamp) and hydrogen peroxide (HUVA therapy), with special emphasis on leprosy. Med. Hypotheses **57** (2001) 484–486.

Despite its abundance and certain therapeutic value, the importance of sunlight in the treatment of infectious skin diseases has not been fully exploited. One reason is that a sufficient amount of the damaging components of sunlight (UVC and most UVB) cannot reach us and the band of UV that can reach (UVA) is a poor inactivator of living cells. UVA, however, can be deleterious to cells in the presence of sensitizers and a number of biological and chemical sensitizers have been identified which can inactivate microbes in the presence of UVA. Of several known agents, I have selected hydrogen peroxide (H_2O_2) as a UVA sensitizer and propose that a combined action of H_2O_2 and UVA (HUVA therapy) can be utilized in controlling skin infections of various types. Of particular interest is infection by *Mycobacterium leprae,* which is known to affect many millions of humans globally. H_2O_2 being relatively cheap (and UVA from the sun being free) the cost of application, particularly in Third-World countries where leprosy is more common, would be low and, therefore, the treatment an be employed on a wide scale. A further reason for proposing the use of H_2O_2 is that, out of several agents we have tested, this was found to be the most potent; it is also easily able to reach target sites, very cheap, relatively safe and there is no known microbial resistance to HUVA.—Author's Abstract

Goto, M. Chemotherapy of leprosy: theoretical basis of new guideline in Japan. Nihon Hansenbyo Gakkai Zasshi **70** (2001) 151–155.

For the effective treatment of leprosy, we should consider that 1) more time is needed for the elimination of bacilli than ordinary bacterial infection, 2) bactericidal therapy often induces host immunity called reactions, 3) rapid treatment is needed for the reactions. Last year, *ad hoc* committee of Japanese Leprosy Association recommends standard treatment protocol of leprosy in Japan, which is a modification of the World Health Organization's multidrug therapy. For multibacillary (MB) with bacterial index (BI> or = 3) before treatment, 2 years treatment by rifampin, dapsone and clofazimine (MDT/MB) is necessary. When BI decrease is not satisfactory (BI value decrease <2 steps, or final BI> or = 3) after 2 years, MDT/MB should be continued until BI negativity and loss of active lesions. Theoretical background of our proposal is describe.—Author's Abstract

Kaluarachchi, S. I., Fernandopulle, B. M. R. and Gunawardane, B. P. Hepatic and haematological adverse reactions associated with the use of multidrug therapy in leprosy—a five year retrospective study. Indian J. Lepr. **73** (2001) 121–129.

This study analyzes retrospectively some of the risks associated with the use of WHO-multidrug therapy (MDT) in Sri Lanka. Case records of 3,333 new cases of leprosy attending the Central Leprosy Clinic in Colombo during 1991–1995, were analyzed for adverse drug reactions involving the liver and blood. There were 81 reports of suspected hepatic or hemotological adverse reactions associated with the use of MDT, of which 39 were classified as hemolytic anemia, 25 as toxic hepatitis, 2 as methemoglobinemia and 15 as anemia. Dapsone, was incriminated in the majority of adverse reactions (72%). Adverse drug reactions were more common in female than male subjects (55% vs 45%; P <0.5), but there was no significant differences between the age groups. The majority of adverse reactions were seen within the first three months of initiation of MDT. This study in no way undermines the benefits of MDT but highlights the risks and suggests measures to minimize these risks.—Authors' Abstract

Li, H. Y., Ran, S. P., Weng, X. M., Li, T. G., Deng, X. H. and Li, F. T. Relapses in leprosy patients treated with rifampicin plus dapsone after varying periods of dapsone monotherapy. Indian J. Lepr. **73** (2001) 1–10.

During 1994–96, a survey was conducted among former leprosy patients from Wenshan Prefecture, Yunnan, China, to determine the occurrence of relapses and rifampin resistance. These patients (531 patients with multibacillary (MB) leprosy, 313 patients with paucibacillary (PB) leprosy) were all treated with dapsone monotherapy followed by combined therapy with rifampin and dapsone between 1978 and 1985. Relapse rate was significantly low for the 482 MB patients receiving >12 months combined therapy, as compared with the 49 MB cases receiving <12 months of combined therapy ($p = 0.0000003$). The relapse rate was also related to the duration of dapsone monotherapy prior to combined therapy. The difference in relapse rate in 247 PB patients following >12 months of combined therapy was also statistically significant, as compared with the relapse rate 66 PB cases who had received <12 months combined therapy ($p = 0.0014$). Five strains of *Mycobacterium leprae* isolated from relapsed patients were sensitive to rifampin by mouse foot pad test. All relapsed patients responded favorably to fixed duration multidrug therapy (MDT) regimen for MB cases.—Trop. Dis. Bull.

2–3 Lesion Multicentre Trial Group. A comparative trial of single dose chemotherapy in paucibacillary leprosy patients with two to three skin lesions. Indian J. Lepr. **73** (2001) 131–143.

A multicentric, double-blind, controlled, clinical trial was carried out to compare the efficacy of a combination of rifampin 600 mg plus ofloxacin 400 mg plus minocycline 100 mg (ROM) administered as single dose with that of standard WHO/MDT/PB six-month regimen. The study subjects were 236 previously untreated, smear-negative patients, without nerve trunk involvement and having only two or three skin lesions. Randomization was done on individual patient basis. Results were analyzed for mean clinical score for improvement, marked clinical improvement and complete clinical cure at the time of release from treatment and at 12 months and 18 months of follow-up.

Clinical improvement was seen in most patients in both regimens. Marked improvement (i.e., more than 90% reduction in clinical score) at 18 months was seen in 46.2% and 53.4% of the patients treated with ROM and standard regimens, respectively. But a significant difference in favor of standard PB regimen was seen in patients with three skin lesions and in patients where more than one body part was affected. Reversal reaction and adverse drug reactions were minimal in both groups.—Authors' Abstract

Clinical Sciences

Asako Ishikawa, Satoshi Ishikawa and Masahisa Hirakawa. Osteoporosis, bone turnover and hypogonadism in elderly men with treated leprosy. Lepr. Rev. **72** (2001) 322–329.

In male hypogonadism associated with bone loss, it is important to determine whether bone loss continues with aging and an increased risk of fracture. We studied bone metabolism in 86 male leprosy patients who were classified according to the presence or absence of osteoporosis. Osteoporosis was present when men had lumbar compression fractures or a mean BMD-2SD that of normal Japanese men in each age decade. Four men had fractures. Serum concentrations of 1,25-dihydroxyvitamin D and high-sensitivity parathyroid hormone were almost normal in both groups, whereas free testosterone and estradiol were significantly lower in the osteoporosis group than in the non-osteoporosis group (free testosterone: $p < 0.01$, estradiol: $p < 0.05$). The urinary concentrations of pyridinoline and deoxypyridinoline, as a marker of bone absorption, were significantly higher in the osteoporosis group than in the non-osteoporosis group (pyridinoline: $p < 0.01$, deoxypyridinoline: $p < 0.01$). The serum concentration of osteocalcin, a marker of bone formation, was significantly higher in the osteoporosis group than in the non-osteoporosis group ($p < 0.01$). Elevated concentration means that bone repair is increased possibly because of compensation mechanisms for increased bone loss. In the osteoporosis group, hypogonadism occurred, and high bone turnover continued even in older men. We recommend clinical studies of treatment, such as replacement therapy, to prevent bone loss and increasing risk of fractures in older men with leprosy.—Authors' Abstract

Azulay, R. D., Mendonca, I. R., Santos, C. M., Monteiro, P. C., Lazera, M. S., Kac, B. K. and Schechtman, R. C. Cutaneous cryptococcosis associated with lepromatous leprosy. Int. J. Dermatol. **40** (2001) 412–414.

A 65-year-old Brazilian man was presented with an erythematous nodular lesion on the left forearm. The patient had been treated with multidrug therapy for 8 months for lepromatous leprosy. During therapy, he developed recurrent episodes of reactions which were treated with high doses of prednisone and thalidomide. The histopathology of the cutaneous nodular lesion showed a granulomatous inflammatory infiltrate; some histiocytes contained vacuolations and others demonstrated oval-like or coma-like structures. The specimen was cultivated in Sabouraud agar at room temperature. The colonies were transferred to petri dishes containing Niger Seed Agar (NSA). The confirmed diagnosis was *Cryptococcus neoformans* var. neoformans based on microscopy and physiology, including the canavanine-glycine-bromothymol blue (CGB) medium (Lazera MS, Pires FDA, Camillo-Coura L, et al. Natural habitat of *Cryptococcus neoformans* var. neformans in decaying wood forming hollows in living trees. J. Med. Vet. Mycol. 34 1996 127–131). The liquor culture was negative. Hemoculture and urine culture were also negative. A latex agglutination test was blood positive and liquor negative. The patient's hemogram revealed normocytic anemia and normal total and differential white blood count. The CD4 count was $189/m^3$ and the CD8 count was $141/m^3$. Serology for anti-human immunodeficiency virus-I (anti-HIV-I) antibodies was negative. The X-ray of the lungs showed an areolar image in the superior lobe of the right lung. Therapy with prednisone was suspended and fluconazole (300 mg/day) was prescribed. The nodular cutaneous lesion regressed completely after 90 days. The patient was submitted to a second skin biopsy for treatment control. The culture of the specimen taken was still positive and the histopathology showed the same picture as before treatment. After 5 months of continued therapy with fluconazole, another biopsy was performed, but no fungus was recovered from the specimen.—Authors' Abstract

Bhushan, K., Inderjeet, K., Ranju, R., Mandal, S. K. and Sharma, V. K. Involvement of male genitalia in leprosy. Lepr. Rev. **72** (2001) 70–77.

Four hundred sixty-seven male leprosy patients attending a leprosy clinic in Chandigarh, India between March 1993 and July 1999 were screened for genital involvement. Genital lesions were observed in 6.6% of all male cases of leprosy. They were seen most frequently in lepromatous leprosy (25.8%), followed by borderline lepromatous (13.3%) and borderline tuberculoid (1.4%) leprosy.—Trop. Dis. Bull.

Chen Shumin, Zhang Lin, Wang Zhaozi, Zhou Jiyang, Liu Yingwei and Mao Chengyu. Experiences from a collaborative project on the prevention of disability in leprosy patients in Shandong Province, the People's Republic of China. Lepr. Rev. **72** (2001) 330–336.

Shandong Province (present population 89 million) in the People's Republic of China established a leprosy control program in 1955. Between that year and the end of 1999, allowing for death and migration, the cumulative number of cases registered was 53,618, including 120 cases on multidrug therapy (MDT) and 18,248 who had completed satisfactory courses of dapsone monotherapy and/or MDT. Of this latter group, 9500 cases (52%) suffered from visible disabilities (grade 2 of the WHO classification). Prevalence and incidence rates of leprosy have decreased dramatically since 1955 and, on average, only 50–70 new cases are now being detected annually in the entire province. Leprosy is thus no longer a public health problem, but the existence of such a large number of patients with grade 2 disabilities is clearly a matter of serious concern. This paper describes a pilot project to investigate the potential of health personnel in the leprosy program and the dermatology and sexually transmitted diseases services to a) prevent deterioration of existing disabilities in ex-patients through self-care and b) prevent new neuritis in patients on MDT through early detection and the use of steroids.—Authors' Abstract

Illarramendi, X., Carregal, E., Nery, J. A. and Sarno, E. N. Progession of acral bone resorption in multibacillary leprosy. Acta Leprol. **12** (2000) 29–37.

Although leprosy became a curable disease after implementation of the Global Strategy for the Elimination of Leprosy (WHO), mutilations and deformities are still commonplace in endemic countries. Hence, it remains important to evaluate the prevalence rate and the risk factors of acral bone resorption in the multidrug therapy (MDT) era. A cohort of 105 newly-diagnosed adult multibacillary leprosy patients admitted for treatment between 1990–1992 was surveyed until 1999. Progression of bone resorption (BR) in cured leprosy patients was observed up to 8 years after release from MDT. Twenty-three percent of the patients were found to have acral resorption. BR was found to be associated with the male sex, the grade of disability at diagnosis with other deformities and with the occurrence of four or more lepra reactions. Patient surveillance after release from MDT continues to be a necessary procedure in individuals with disabilities and recurrent or persistent reactions.—Authors' Abstract

Lee, S. B., Kim, S. K., Kang, T. J., Chae, G. T., Chun, J. H., Shin, H. K., Kim, J. P., Ko, Y. H. and Kim, N. H. The prevalence of *folP1* mutations associated with clinical resistance to dapsone, in *Mycobacterium leprae* isolates from South Korea. Ann. Trop. Med. Parasitol. **95** (2001) 429–432.

The isolates were collected from 50 leprosy patients from the Korea Republic, who had each shown a persistent or increased bacterial index (BI) in the skin smears produced during or after routine treatment with dapsone or multidrug therapy [date not given]. A dapsone susceptible strain of *M. leprae,* Thai-53, was used as a control. The *M. leprae*-DNA was isolated and then examined in a polymerase chain reaction-single strand conformation polymorphisms (PCR-SSCP) assay, using two primers that amplified a *folP1* fragment containing sulphone-resistance-determining region. Seventeen (34%) of the 50 South Korean isolates analyzed showed SSCP-banding profiles that differed from that of dapsone-susceptible *M. leprae,* Thai-53. Sequence analysis of the DNA fragments of the 17 isolates showing mutation re-

vealed *folP1* fragments containing 17 missense mutations and one silent mutation. Ten (59%) of the 17 isolates had an A to G transition at nucleotide 157, resulting in the substitution of an alanine for a threonine in codon 53. Five others (33%) had a C to G transversion at nucleotide 164, resulting in the substitution of an arginine for a proline, one (7%) had a C to T transition at nucleotide 163, resulting in the substitution of a serine for a proline, and one (7%) had a double mutation: a G to A transition at nucleotide 162; and a C to G transversion at nucleotide 164. Overall, the prevalences of mutations observed indicate that mutations in codons 53 or 55 of *M. leprae folP1* are responsible for at least some of the dapsone resistance in South Korea.—Trop. Dis. Bull.

LeMaster, J. W., John, O. and Roche, P. W. 'Jhum-jhum'—a common paraesthesia in leprosy. Lepr. Rev. **72** (2001) 100–101.

A retrospective study of 277 records of previously untreated leprosy patients was conducted to determine the frequency of paraesthesia ("jhum-jhum") among these patients. All patients had enrolled for multiple drug therapy treatment at Anandaban Leprosy Hospital, Kathmandu, Nepal, between 1993 and 1995, and had regularly attended the outpatient department for an average of 30 months. Out of 277 participants, 107 (39%) recorded episodes of jhum-jhum. Most jhum-jhum was manifested as burning and tingling sensations (47%) or neuropathic pain alone or in combination with burning and tingling (45%). Symptoms were more commonly reported in the extremities (hands only [25%], feet only [20%], both hands and feet [33%]) than in the face (4%) or whole body (1.8%). The majority of patients (72%) complained of jhum-jhum in limbs with leprosy-affected nerve supply. The symptom occurred in leprosy patients before (15%), during (61%) and after (24%) anti-leprosy treatment. Two-thirds of the patients experienced jhum-jhum as episodic symptoms. There was an association between the frequency of reported jhum-jhum and age, reflecting an increase of paraesthesia in older persons in general. No apparent association was evident with gender or with type of leprosy disease or with the occurrence of lepromatous reactions. From these observations, it can be concluded that paraesthesia is a common complaint among leprosy patients.—Trop. Dis. Bull.

Salazar, J. J., Serrano, G. G., Leon-Quintero, G. I. and Torres-Mendoza, B. M. Use of topical ketanserin for the treatment of ulcers in leprosy patients. Indian J. Lepr. **73** (2001) 103–110.

A comparative study was carried out in which 66 leprosy patients with ulcers were randomly divided in two groups of 33 patients each. Group A (experimental group) was treated with ketanserin gel (2%) and group B with clioquinol cream and/or Lassar paste during a three-month period. At the end of the study, when ulcer sizes in the two groups were compared, the group treated with topical ketanserin showed superior results (p <0.001 using Kolmogorov-Smirnov's test). We conclude that the drug is useful as coadjuavant treatment for healing ulcers in these patients.—Authors' Abstract

Shaw, I. S., Ebenezer, G., Babu, B. and Rao, G. S. Borderline tuberculoid leprosy of the scalp. Lepr. Rev. **72** (2001) 357–361.

A case of borderline tuberculoid leprosy involving the hairy scalp is reported. To the best of our knowledge, only two paucibacillary leprosy patients with scalp lesions have been reported, and in only one was the scalp covered with hair.—Authors' Abstract

Wafaa Ramadan, Basma Mourad, Wael Fadel and Enas Ghoraba. Clinical, electrophysiological, and immunopathological study of peripheral nerves in Hansen's disease. Lepr. Rev. **72** (2001) 35–49.

Hansen's disease is a disease of peripheral nerves. Some patients develop peripheral neuropathy before the diagnosis of the disease, and others develop these complications after starting therapy. Electrophysio-

logical (EP) studies were carried out in Hansen's disease patients. This work studied the neural deficits, electromyography (EMG) and motor nerve conduction (MNC) variables in different types of leprosy and the immunopathology of sural nerve tissue in patients with severe neural deficits. Forty leprosy patients had neurological examinations and an EP study. Histopathological and immunopathological study of sural nerve biopsy specimens was performed for 10 patients with severe neural deficits. The results of the neurological study showed that there was involvement of cranial nerves, muscular system, motor reflexes and sensory system, and trophic and vasomotor changes. EP study showed significant changes in EMG of abductor digiti minimi in patients as compared to controls. MNC variables of common peroneal nerve were abnormal in 80% of all patients, MNC of median nerve was abnormal in 72.5%, while MNC of ulnar nerve was abnormal in 70% and SNC of ulnar nerve was abnormal in 77.5%. In conclusion, electrophysiological investigations have an important role in the detection of muscle denervation and neuropathic changes in leprosy patients, and are safe, rapid and non-invasive. On the other hand, immunopathological study revealed that the degree of immune positivity correlated with the degree of nerve fibrosis.—Trop. Dis. Bull.

Immuno-Pathology

Fairbairn, I. P., Stober, C. B., Kumararatne, D. S. and Lammas, D. A. ATP-mediated killing of intracellular mycobacteria by macrophages is a p2x (7)-dependent process inducing bacterial death by phagosome-lysosome fusion. J. Immunol. **167** (2001) 3300–3307.

Mycobacterium tuberculosis survives within host macrophages by actively inhibiting phagosome fusion with lysosomes. Treatment of infected macrophages with ATP induces both cell apoptosis and rapid killing of intracellular mycobacteria. The following studies were undertaken to characterize the effector pathway(s) involved. Macrophages were obtained from p47(phox) and inducible NO synthase gene-disrupted mice (which are unable to produce reactive oxygen and nitrogen radicals, respectively) and P2X (7) gene-disrupted mice. RAW murine macrophages transfected with either the natural resistance-associated macrophage protein gene 1 (Nramp1)-resistant or Nramp1-susceptible gene were also used. The cells were infected with bacille Calmette-Guerin (BCG), and intracellular mycobacterial trafficking was analyzed using confocal and electron microscopy. P2X (7) receptor activation was essential for effective ATP-induced mycobacterial killing, as its bactericidal activity was radically diminished in P2X (7) (-/-) macrophages. ATP-mediated killing of BCG within p47 (phox-/-), inducible NO synthase (-/-), and Nramp (s) cells was unaffected, demonstrating that none of these mechanisms have a role in the ATP/P2X (7) effector pathway. Following ATP stimulation, BCG-containing phagosomes rapidly coalesce and fuse with lysosomes. Blocking of macrophage phospholipase D activity with butan-1-ol blocked BCG killing, but not macrophage death. ATP stimulates phagosome-lysosome fusion with concomitant mycobacterial death via P2X (7) receptor activation. Macrophage death and mycobacterial killing induced by the ATP/P2X (7) signaling pathway can be uncoupled, and diverge proximal to phospholipase D activation.—Authors' Abstract

Fine, P. E. M., Floyd, S., Stanford, J. L., Nkhosa, P., Kasunga, A., Chaguluka, S., Warndorff, D. K., Jenkins, P. A., Yates, M. and Ponnighaus, J. M. Environmental mycobacteria in northern Malawi: implications for the epidemiology of tuberculosis and leprosy. Epid. Infect. **126** (2001) 379–387.

More than 36,000 individuals living in rural Malawi were skin tested with anti-

gens derived from 12 different species of environmental mycobacteria. Most were simultaneously tested with RT23 tuberculin, and all were followed up for both tuberculosis and leprosy incidence. Skin test results indicated widespread sensitivity to the environmental antigens, in particular to *Mycobacterium scroulaceum, M. intracellulare* and one strain of *M. fortuitum.* Individuals with evidence of exposure to 'fast growers' (i.e., with induration to antigens from fast growers which exceeded their sensitivity to tuberculin), but not those exposed to 'slow growers', were at reduced risk of contracting both tuberculosis and leprosy, compared to individuals whose indurations to the environmental antigen were less than that to tuberculin. This evidence for cross protection from natural exposure to certain environmental mycobacteria may explain geographic distributions of mycobacterial disease and has important implications for the mechanisms and measurement of protection by mycobacterial vaccines.—Trop. Dis. Bull.

Kimura, T. A morphological study of nerve biopsies in leprous neuropathy. Nihon Hansenbyo Gakki Zasshi **70** (2001) 141–144. (in Japanese)

Peripheral nerve biopsies from 10 leprosy patients (6 tuberculoid patients and 4 lepromatous patients) were studied from the morphological aspect. Light microscopical examination showed that the perineurium was markedly thickened by infiltrated cells in tuberculoid type and *Mycobacterium leprae* in lepromatous type. Schwann's cells were markedly decreased in number, and nerve fiber disappeared without regeneration in severe cases. In mild cases, subperineurial edema was present. The nerve fiber density was normal or mildly decreasing. Ultrastructural examination showed abnormality of basal lamina on perineurial cells. The basal lamina of the perineurium completely disappeared in severe cases, and showed splitting, even if the perineurium had normal structure in light microscopy. Both types of leprous neuropathy had same pathological changes in regard to abnormality of the basal lamina. There were many *M. leprae* presented in Schwann's cells, fibroblasts and perineurial cells on the nerve of lepromatous patients, although few *M. leprae* were in the nerve of tuberculoid patients. This study provides that the abnormality of perineurium is characteristic in both types of leprous neuropathy.—Author's Abstract

Kusner, D. J. and Barton, J. A. ATP stimulates human macrophages to kill intracellular virulent *Mycobacterium tuberculosis* via calcium-dependent phagosome-lysosome fsion. J. Immunol. **167** (2001) 3308–3315.

Advances in therapy for tuberculosis will require greater understanding of the molecular mechanisms of pathogenesis and the human immune response in this disease. Exposure of *Mycobacterium tuberculosis*-infected human macrophages to extracellular ATP (ATP(e)) results in bacterial killing, but the molecular mechanisms remain incompletely characterized. In this study, we demonstrate that ATP(e)-induced bactericidal activity toward virulent *M. tuberculosis* requires an increase in cytosolic Ca (2+) in infected macrophages. Based on our previous work with primary infection of human macrophages, we hypothesized that the Ca(2+) dependence of ATP-induced killing of intracellular *M. tuberculosis* was linked to promotion of phagosome-lysosome fusion. Using confocal laser-scanning microscopy, we demonstrate that ATP(e) induces fusion of the *M. tuberculosis*-containing phagosome with lysosomes, defined by accumulation of three lysosomal proteins and an acidophilic dye. Stimulation of phagosome-lysosome fusion by ATP(e) exhibited distinct requirements for both Ca (2+) and phospholipase D and was highly correlated with killing of intracellular bacilli. Thus, key signal transduction pathways are conserved between two distinct models of human macrophage antituberculous activity: primary infection of naive macrophages and physiologic stimulation of macrophages stably infected with *M. tuberculosis.*—Authors' Abstract

Le Van Truoc, Ho Minh Ly, Nguyen Kim Thuy, Dang Due Trach, Stanford, C. A. and Stanford, J. L. Vaccination against leprosy at Ben San Leprosy Centre, Ho Chi Minh City, Vietnam. Vaccine **19** (2001) 3451–3458.

Three vacccines, BCG alone, BCG + 10^7 killed *Mycobacterium vaccae* and 10^8 killed *M. vaccae* alone, were studied in children living in close contact with leprosy. In the year before vaccination, 14/446 (3.1%) children had developed leprosy. Among those who were not vaccinated, 9/74 (12.2%) developed the disease in the first four years of the study and 5/65 (7.7%) developed the disease in the second four years. In comparison with this, among those vaccinated, 20/343 (5.8%) developed leprosy in the first four years and 5/323 (1.5%) developed leprosy in the second 4 years. This represents 52.5% protection in the first four years and 80.5% in the second four years. There were no significant differences in protection afforded by each of the three vaccines but the success of the killed preparation of *M. vaccae* is an important finding.—Trop. Dis. Bull.

Moraes, M. O., Duppre, N. C., Suffys, P. N., Santos, A. R., Almeida, A. S., Nery, J. A. C., Sampaio, E. P. and Sarno, E. N. Tumor necrosis factor-α promoter polymorphism TNF2 is associated with a stronger delayed-type hypersensitivity reaction in the skin of borderline tuberculoid leprosy patients. Immunogenetics **53** (2001) 45–47.

This study investigated the correlation between the –308 tumor necrosis factor-α promoter polymorphic allele (TNF2 genotype) and the granulomatous response to lepromin in 74 borderline tuberculoid leprosy patients from the Leprosy Out-Patient Unit of the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil). Lepromin challenge was performed at the time of diagnosis (active disease) by intradermically injecting in the volar surface of the left forearm, 0.1 ml of lepromin A containing 1.6×10^8 heat-killed bacilli/ml. Mitsuda reactions were recorded 28 days after inoculation and were coded as a four-class categorical trait (<3, 3–5, 6–9, and >10). The Mitsuda reactions were analyzed according to the TNF promoter genotype, the presence or absence of BCG vaccination, and the occurrence of reversal reaction. The frequency distribution of the observed Mitsuda reaction considered as a four-class categorical trait demonstrated that 82.2% of the patients carrying the TNF2 allele presented a ≥6-mm skin test induration when compared to non-TNF2 carriers (54.3%, χ^2=4.29, $p < 0.05$). Furthermore, TNF2 carriers presented a Mitsuda reaction of 7.45±3.47 mm, a result of borderline significance ($p = 0.057$, data not shown). Results suggest that TNF-α plays a crucial role in the immunological response against *Mycobacterium leprae*.—Trop. Dis. Bull.

Nomaguchi, H., Jahan, N., Mandal, B. C., Yogi, Y., Kawatsu, K., Yoshizawa, Y., Okamura, H. and Makino, M. IL-12 and IL-18 synergistically induce the bactericidal activity of murine peritoneal cells against *M. leprae*. Nihon Hansenbyo Gakkai Zasshi **70** (2001) 113–119.

We examined the effect of IL-12 and IL-18 on bactericidal activities of mouse peritoneal cells (PC) against *Mycobacterium leprae*. We demonstrated that IL-12 and IL-18 synergistically induced the NO-dependent bactericidal activity of PC by stimulating Natural Killer (NK) cells and T-cells through IFN-gamma production. IL-12 and IL-18 induced host cell death through NK-cells and T-cells. Therefore, IL-12 and IL-18 play an important role on direct killing of intracellular *M. leprae* and on indirect killing of them through inducing host cell death.—Authors' Abstract

Ohyama, H., Matsushita, S., Nishimura, F., Kato, N., Hatano, K., Takashiba, S. and Murayama, Y. T cell responses to major membrane protein II (MMPII) of *Mycobacterium leprae* are restricted by HLA-DR molecules in patients with leprosy. Vaccine **20** (2001) 475–482.

Major membrane protein II (MMP II) of *Mycobacterium leprae* (*M. leprae*) is a 22-kDa protein inducing humoral immune response in leprosy patients. MMP II-specific bulk T cell lines were established from leprosy patients to determine major T cell epi-

topes in MMP II and to evaluate lymphokine production induced by MMP II. These bulk T cell lines reacted to one or more peptides in the locus of amino acid residues from 23 to 109 of MMP II. The proliferative responses of all T cell lines were mainly inhibited by the addition of anti-DRB1 mAb. Many bulk T cell lines induced IFN-gamma, IL-5, but not IL-4. However, it was not possible to distinguish the LL or TT types of leprosy based on the pattern of T cell epitopes and the lymphokine productivity in the responses against MMP II. Thus, it appears that T cell response to MMP II is restricted by the HLA-DRB1 molecule, but not by DQ and DP molecules, which results in the induction of IFN-gamma production.—Authors' Abstract

Oliveira, M. M., Charlab, R. and Pessolani, M. C. *Mycobacterium bovis* BCG but not *Mycobacterium leprae*-induces TNF-alpha secretion in human monocytic THP-1 cells. Mem. Inst. Oswaldo Cruz **96** (2001) 973–978.

In this study, we compared the level of TNF-alpha secretion induced in monocytic THP-1 cells after phagocytosis of *Mycobacterium leprae,* the causative agent of leprosy, and *M. bovis* BCG, an attenuated strain used as a vaccine against leprosy and tuberculosis. The presence of *M. leprae* and BCG was observed in more than 80% of the cells after 24 h of exposure. However, BCG but not *M. leprae* was able to induce TNF-alpha secretion in these cells. Moreover, THP-1 cells treated simultaneously with BCG and *M. leprae* secreted lower levels of TNF-alpha compared to cells incubated with BCG alone. *M. leprae* was able, however, to induce TNF-alpha secretion both in blood-derived monocytes as well as in THP-1 cells pretreated with phorbol myristate acetate. The inclusion of streptomycin in our cultures, together with the fact that the use of both gamma-irradiated *M. leprae* and heat-killed BCG gave similar results, indicate that the differences observed were not due to differences in viability but in intrinsic properties between *M. leprae* and BCG. These data suggest that the capacity of *M. leprae* to induce TNF-alpha is dependent on the stage of cell maturation and emphasize the potential of this model to explore differences in the effects triggered by vaccine strain versus pathogenic species of mycobacteria on the host-cell physiology and metabolism.—Authors' Abstract

Singh, P. A., Ranjan Agarwal, Vatsala Misra, Gupta, S. C. and Bajaj, A. K. Clinicohistopathological concordance in leprosy. Trop. Doct. **30** (2000) 228–231.

Histopathological.examination of biopsies from 111 patients with clinically diagnosed leprosy was carried out in order to observe the clinico-histopathological correlation [India]. Clinical diagnosis was based on Ridley and Jopling (R–J) classification and World Health Organization (WHO) classification. The concordance rate between the 2 clinical classifications was 73.8%. Sections were stained with hematoxylin and eosin (H&E) and Ziehl-Neelsen (Z–N) stains. The histological classification was as per the R–J criteria. Skin biopsy showed evidence of leprosy in 104 cases (93.69%). Overall concordance was observed in 58.6% (R–J) and 85.6% (WHO classification). The kappa test, when applied, showed significant agreement between clinical and histopathological diagnosis ($z = 11.775$; $p < 0.001$). Individual subtypes showed variable concordance rates—which were again higher using WHO classification. When some of the subtypes were combined, the concordance rate was 83.02% for TT+BT; 72.58% for BT+BB+BL; 73.91% for BL+LL; 80.77% for BL+HL and 100% for LL+HL. The present study highlights the importance of histopathological examination for exact subtyping of leprosy, so as to facilitate the institution of accurate mode of therapy and regular follow-up of patients to prevent undesirable complications.—Trop. Dis. Bull.

Suneetha, S., Arunthathi, S. Kurian, N. and Chacko, C. J. Histological changes in the nerve, skin and nasal mucosa of patients with primary neuritic leprosy. Acta Leprol. **12** (2000) 11–18.

Primary neuritic leprosy (PNL) presents as a peripheral neuropathy with no visible

skin patches and skin smears negative for acid-fast bacilli. The pathogenesis of PNL is poorly understood. The aim of the study was to document the histological changes in the nerve, apparently normal skin and nasal mucosa in PNL and to study its significance to the pathogenesis of leprosy lesions. The study is based on a cohort of 208 PNL patients registered at the Schieffelin Leprosy Research and Training Centre, Karigiri. All patients had a nerve biopsy, 196 had a skin biopsy and 39 had a nasal mucosal biopsy. The findings reveal that PNL patients exhibit a spectrum of disease histologically in the nerve ranging from lepromatous to tuberculoid leprosy with a significant proportion (46%) manifesting a multibacillary leprosy histology. Findings in the apparently normal skin and nasal mucosa reveal that there are widespread changes due to leprosy in tissues such as the skin and nasal mucosa even when the disease appears clinically confined to a few nerves. PNL may be an early stage in the pathogenesis of the disease before the appearance of skin lesions. The number of nerves enlarged and lepromin status did not give any clue to the nature of underlying disease.—Authors' Abstract

Microbiology

Chakrabarty, A. N., Dastidar, S. G., Parthajit-Banerjee, A. S. and Roy, R. Leprosy bacillus—possibly the first chemoautotrophic human pathogen cultivated *in vitro* and characterised. Indian J. Exp. Biol. **39** (2001) 962–983.

Leprosy bacillus (LB) and leprosy derived *in vitro* culture forms, the chemoautotrophic nocardioform (CAN) bacteria showed an extremely close homology and identity with each other as regards a chemoautotrophic nutritional pattern, a nocardioform norphology, a weak acid-fastness coupled with Gram and Gomori's stain positivity, an exclusive mycolate and lipid profile, a phenolic glycolipid (PGL-1) and a highly sequestrated DNA characteristic, namely a unique small size, a low G+C % mole, an exceptionally high γ and UV radiation resistance, and a high thermal resistance. LB/CAN bacteria (CANb) gave positive signals for 36-kDa protein PCR, as well as for 65-kDa epitope, and hybridization with two or more probes and also RFLP analysis. Both LB and CAN bacteria exhibited bacillary multiplication in the mouse foot pads (MFP), nerve infiltration and evidences for local pathogenicity associated with pronounced systemic invasion. A highly reproducible mutilation model could be established which enabled a successful application of the postulates of Koch. The proof of their anergic reactions in LL cases counterpoised against Mitsuda-type strong nodular responses, mirroring the total reactions of leprosy bacilli in TT cases, in accordance with the dictum of XIth International Leprosy Congress (1978). Thus, the chemoautotrophic nutritional requirements of LB, entirely unsuspected for a medically important pathogenic bacterium, having dimorphic (both bacillary and mycelial) characters with spores, mycelia and granules and unique pathogenicity of mutilation manifested through the virulence factor, the enzyme collagenase, made LB or *M. leprae* the highly enigmatic bacterium for so long.—Authors' Abstract

Gey van Pittius, N. C., Gamieldien, J., Hide, W., Brown, G. D., Siezen, R. J. and Beyers, A. D. The ESAT-6 gene cluster of *Mycobacterium tuberculosis* and other high G+C Gram-positive bacteria. Genome Biol. **2** (2001) RESEARCH0044.

Background: The genome of *Mycobacterium tuberculosis* H37Rv has five copies of a cluster of genes known as the ESAT-6 loci. These clusters contain members of the CFP-10 (1hp) and ESAT-6 (esat-6) gene families (encoding secreted T-cell antigens that lack detectable secretion signals) as well as genes encoding secreted, cell-wall-associated subtilisin-like serine proteases, putative ABC transporters, ATP-binding proteins and other membrane-associated proteins. These membrane-associated and energy-providing proteins may function to

secrete members of the ESAT-6 and CFP-10 protein families, and the proteases may be involved in processing the secreted peptide. Results: Finished and unfinished genome sequencing data of 98 publicly available microbial genomes has been analyzed for the presence of orthologs of the ESAT-6 loci. The multiple duplicates of the ESAT-6 gene cluster found in the genome of *M. tuberculosis* H37Rv are also conserved in the genomes of other mycobacteria, for example *M. tuberculosis* CDC1551, *M. tuberculosis* 210, *M. bovis, M. leprae, M. avium,* and the avirulent strain *M. smegmatis.* Phylogenetic analyses of the resulting sequences have established the duplication order of the gene clusters and demonstrated that the gene cluster known as region 4 (Rv3444c–3450c) is ancestral. Region 4 is also the only region for which an ortholog could be found in the enomes of *Corynebacterium* diphtheriae and *Streptomyces coelicolor.* Conclusions: Comparative genomic analysis revealed that the presence of the ESAT-6 gene cluster is a feature of some high-G+C Gram-positive bacteria. Multiple duplications of this cluster have occurred and are maintained only within the genomes of members of the genus *Mycobacterium.*—Authors' Abstract

Nakamura, M. Reasons why *Mycobacterium leprae* cells do not multiply under the cell-free condition. Nihon Hansenbyo Gakkai Zasshi **70** (2001) 127–133. (in Japanese)

Our previous paper reported that the intracellular ATP content in cells of *M. leprae* consistently increased in the medium containing adenosine after 4–6 weeks of cultivation and decreased thereafter. The reason why ATP generation ceased 4–6 weeks after cultivation is not clear, but it was determined that the termination in ATP generation was not a result of deterioration in the culture medium during cultivation because a renewal trial of the old culture medium by freshly prepared culture medium had no effect or further maintenance or progressive increase in ATP generation. From the results obtained in a renewal trial of the culture medium, I would like to speculate that the reason why *M. leprae* cells do not multiply *in vitro* might be due to the characteristic property of the cell wall of *M. leprae,* i.e., fragility.—Author's Abstract

Nakata, N. Genome features of *Mycobacterium leprae.* Nihon Hansenbyo Gakkai Zasshi **70** (2001) 135–140. (in Japanese)

Recent studies have revealed that the *Mycobacterium leprae* genome contains many pseudogenes. This short review summarizes the structural features of the *M. leprae* genome and genes.—Author's Abstract

Tortoli, E., Bartoloni, A., Bottger, E. C., Emler, S., Garzelli, C., Magliano, E., Mantella, A., Rastogi, N., Rindi, L., Scarparo, C., and Urbano, P. Burden of unidentifiable mycobacteria in a reference laboratory. J. Clin. Microbiol. **39** (2001) 4058–4065.

Modern identification techniques at the genomic level have greatly improved the taxonomic knowledge of mycobacteria. In adjunct to nucleic acid sequences, mycobacterial identification has been endorsed by investigation of the lipidic patterns of unique mycolic acids in such organisms. In the present investigation, the routine use of high-performance liquid chromatography (HPLC) of mycolic acids, followed by the sequencing of the 16S rRNA, allowed us to select 72 mycobacterial strains, out of 1,035 screened, that do not belong to any of the officially recognized mycobacterial species. Most strains (i.e., 47) were isolated from humans, 13 were from the environment, 3 were from animals, and 9 were from unknown sources. The majority of human isolates were grown from the respiratory tract and were, therefore, most likely not clinically significant. Some, however, were isolated from sterile sites (blood, pleural biopsy, central venous catheter, or pus). Many isolates, including several clusters of two or more strains, mostly slow growers and scotochromogenic, presented unique genetic and lipidic features. We hope the data reported here, including the results of major conventional identification tests, the HPLC profiles of strains isolated several times, and the whole sequences of the 16S

rRNA hypervariable regions of all 72 mycobacteria, may encourage reporting of new cases. The taxonomy of the genus *Mycobacterium* is, in our opinion, still far from being fully elucidated, and the reporting of unusual strains provides the best background for the recognition of new species. Our report also shows the usefulness of the integration of novel technology to routine diagnosis, especially in cases involving slow-growing microorganisms such as mycobacteria.—Authors' Abstract

Vissa, V. D. and Brennan, P. J. The genome of *Mycobacterium leprae*: a minimal mycobacterial gene set. Genome Biol. **2** (2001) 1023.

Comparison of the recently sequenced genome of the leprosy-causing pathogen *Mycobacterium leprae* with other mycobacterial genomes reveals a drastic gene reduction and decay in *M. leprae* affecting many metabolic areas, exemplified by the retention of a minimal set of genes required for cell-wall biosynthesis.—Authors' Abstract

Williams, D. L., Pittman, T. L., Gillis, T. P., Matsuoka, M. and Kashiwabara, Y. Simultaneous detection of *Mycobacterium leprae* and its susceptibility to dapsone using DNA heteroduplex analysis. J. Clin. Microbiol. **39** (2001) 2083–2088.

Currently recommended control measures for treating leprosy with multidrug therapy should control the spread of drug-resistant strains; however, dapsone (DDS) resistance continues to be reported. Comprehensive estimates of drug-resistant leprosy are difficult to obtain due to the cumbersome nature of the conventional drug susceptibility testing method using mouse footpad inoculation, which requires at least 6 months to obtain results. Recently, it has been determined that DDS-resistant strains contain missense mutations in codon 53 or 55 of the *folP1* gene of *M. leprae*, and definitive evidence linking these mutations with DDS resistance in *M. leprae* has been obtained. Based on these mutations, a heteroduplex DDS *M. leprae* (HD-DDS-ML) assay was developed for the simultaneous detection of *M. leprae* and of its susceptibility to DDS. The assay relies on the PCR amplification of a *M. leprae*-specific 231-bp fragment of *folP1* containing codons 53 and 55. The PCR products are allowed to anneal to a universal heteroduplex generator, and the separation of the resultant DNA duplexes is accomplished by polyacrylamide gel electrophoresis. *M. leprae* was detected in crude cell lysates of skin biopsy specimen homogenates from 8 leprosy patients and from *M. leprae* infected mouse or armadillo tissues infected with 14 separate strains using the HD-DDS-ML assay. These *M. leprae* strains were originally obtained from the Anandaban Leprosy Hospital in Kathmandu, Nepal; the National Hansen's Disease Programs in Baton Rouge, Louisiana, USA; the Schieffelin Leprosy Research and Training Centre in Karigiri, India; and The Leprosy Research Center, National Institute of Infectious Diseases in Tokyo, Japan [date not given]. The assay was specific for *M. leprae* in a comparison with results obtained from 14 species of mycobacteria other than *M. leprae* and four bacterial species known to colonize human skin. The HD-DDS-ML assay detected as few as 100 *M. leprae* organisms present in homogenates of human skin and demonstrated a 93% correlation with DDS susceptibility as determined by both DNA sequencing of *folP1* and mouse footpad susceptibility testing. The HD-DDS-ML assay provides a new tool for the simultaneous detection of *M. leprae* and of its susceptibility to DDS from a single specimen. The assay should prove useful for drug resistance surveillance in leprosy control programs when combined with similar molecular tests developed for other drug resistance markers.—Trop. Dis. Bull.

Epidemiology and Prevention

Freerksen, T., Rosenfeld, M., Depasquale, G., Connici, E. and Gatt, P. The Malta Project–a country freed itself of leprosy. A 27-year progress study (1972–1999) of the first successful eradication of leprosy. Chemotherapy **47** (2001) 309-331.

The successful conclusion of the first leprosy eradication program carried out with combination therapy is reported. This program started in Malta in June 1972. It was based on extensive experimental and clinical studies and was formally concluded on 31 December 1999. No new infections occurred after the start of this 27-year progress report. The youngest patient was 16, and the eldest 83 years old. Of the total of 261 cases in the project, 201 had already received pretreatment [mainly with diaminodiphenylsulfone (DDS)] at the start. Sixty-one cases had no pretreatment. These were predominantly elderly patients who were late in deciding to have treatment. The very long follow-up period totaling 27 years was consistently maintained in order to be able to refute all potential objections empirically, e.g., with regard to relapses at a late stage. Besides the overall symptoms which are typical for the broad picture of leprosy, the involvement of the eyes was very striking (at least 50%). The therapeutic effect was of very rapid onset in these cases without surgery. Rifampin (RMP) + isoniazid + prothionamide + DDS (trade name Isoprodian-RMP) was used as medication in a fixed combination. This fixed combination had already proved to be highly effective in the treatments during the course of the project, surprising therapy results (including lifesaving effects) were also noticed in other diseases.—Authors' Abstract

Gonzalez Ochoa, C. E. and Abreu, A. Leprosy surveillance in low-prevalence situations. Pan Am. J. Pub. Hlth. **9** (2001) 94–101.

The majority of the countries in Latin America have reduced the prevalence of leprosy to less than one case for every 10,000 persons. The next step for these countries is to eliminate the disease at the regional and local level, in "pockets" that still have rates higher than one per 10,000. Given the demographic transition, the existence of areas with high transmission levels, and the necessity for more sensitive indicators, there is a need to change basic strategies, strengthen surveillance systems, and refocus resources. It is important to revamp efforts through tactics, such as, identifying priority geographical areas, customizing interventions, improving indicators and combining passive and active surveillance. This can be done by redesigning surveillance systems to integrate the clinical, laboratory, epidemiological research, and supply components. The results of the process should provide a minimum set of indicators that make it possible to monitor and evaluate the effectiveness and efficiency of action plans for the post-elimination stage.—Trop. Dis. Bull.

Lapa, T., Ximenes, R., Silva, N. N., Souza, W., Albuquerque, Md. M., and Campozana, G. Leprosy surveillance in Olinda, Brazil, using spatial analysis techniques. Cad. Saude Publica. **17** (2001) 1153–1162.

In the State of Pernambuco, Brazil, leprosy has been mainly an urban disease, with an uneven geographical distribution related at least partially to the way urban space has been occupied and transformed. Spatial analysis may thus become an important tool to establish an epidemiological surveillance system for leprosy. Homogeneous micro-areas were defined in the city of Olinda through the integration of two databases, the Population Census and SINAN, and through the use of digital maps and geoprocessing techniques. Census tracts were classified according to a social deprivation index (SDI), and micro-area homogeneity was based on similar values for this indicator. Cluster analysis (K-means) was used to define cut-offs between strata. The same procedure was re-

peated using the income variable only. When the association was tested between the mean SDI value and the mean leprosy detection rate for the period 1991–1996, the value obtained for r2 was 66.1% in the multiplicative model, increasing to 84.3% when the income variable was used. To define different intervention strategies, census tracts were regrouped in three levels of risk: high, moderate, and low. The methodology enabled the identification (within each health district) of groups and/or areas with different risks of leprosy, hence allowing for the definition of control measures.—Authors' Abstract

Murthy, B. N., Subbiah, M., Boopathi, K., Ramakrishnan, M. D. and Gupte, M. D. Lot quality assurance sampling (LQAS) for monitoring leprosy elimination in an endemic district in TamilNadu. Indian J. Lepr. **73** (2001) 111–119.

This paper examines whether the health administration can use lot quality assurance sampling (LQAS) for identifying high prevalence areas for leprosy for initiating necessary corrective measures. The null hypothesis was that leprosy prevalence in the district was at or above ten per 10,000 and the alternative hypothesis was that it was at or below five per 10,000. A total of 25,500 individuals were to be examined with 17 as an acceptable maximum number of cases (critical value). Two-stage cluster sample design was adopted. The sample size need not be escalated as the estimated design effect was 1. During the first phase, the survey covered a population of 4,837 individuals out of whom 4,329 (89.5%) were examined. Thirty-five cases were detected and this number far exceeded the critical value. It was concluded that leprosy prevalence in the district should be regarded as having prevalence of more than ten per 10,000 and further examination of the population in the sample was discontinued. LQAS may be used as a tool by which one can identify high prevalence districts and target them for necessary strengthening of the program. It may also be considered for certifying elimination achievement for a given area.—Authors' Abstract

Xiang-Sheng Chen, Wen-Zhong Li, Cheng Jiang, Cheng-Bin Zhu and Gan-Yun Ye. Studies on mode of detection of leprosy in China during the years 1981–1998. Lepr. Rev. **72** (2001) 302–310.

Along with the nationwide economic reform initiated in the early 1980s and the rapid decrease of leprosy endemic after the implementation of multidrug therapy (MDT), the leprosy program changed from 'vertical' to 'horizontal'. An evolution in the mode of detection of leprosy cases has consequently taken place. Based on the nationwide registration of newly detected cases, the profile of patients at detection has been studied. The proportions of cases corrected significantly with calendar years in detection by dermatological clinics, contact checks, 'clue survey' and mass survey, showing a significant increase in percentage of cases detected through dermatological clinics and contact checks, and decreases through 'clue survey' and mass survey. Detection of cases through dermatological clinics and voluntary reporting have become the main modes of case-finding during 1997–1998, accounting for 37.3% and 28.6%, respectively, where contact check accounts for only 9.1%. In areas with good dermatological services, a significantly higher proportion (75.9%) of cases was detected through dermatological clinics, where voluntary reporting and 'clue survey' were the main modes of detection in endemic areas. As regards confirmation of diagnosis, the great majority of cases were confirmed by leprosy units, even though they were detected in various other situations. Only 6.5% of leprosy cases were detected and subsequently confirmed by doctors in dermatologic clinics. The present modes of detection and their relation to demographical, epidemiological, clinical factors and health services are discussed. This study emphasizes the cardinal importance of the dermatological clinics in the detection of leprosy cases in China at the present time and hence, the need to strengthen the training of doctors in these clinics, while continuously encouraging their involvement in leprosy control.—Authors' Abstract

Rehabilitation

Benbow, C. and Tamiru, T. The experience of self-care groups with people affected by leprosy. Lepr. Rev. **72** (2001) 311–321.

This paper describes the development of self-care groups in Ethiopia by ALERT, and the successes and failures experienced in the process. The groups were started in 1995 in response to two main problems, the increasing number of people dependent on ALERT to heal their wounds despite years of health education, and the limited financial resources of ALERT for wound healing supplies. By December 1999, there were a total of 72 established groups. Group membership was voluntary. There have been a number of positive outcomes. Group members have taken up responsibility for managing and monitoring their own wounds and supplying their own wound healing materials. More attention is paid to their personal hygiene and personal appearance. They also report increased confidence to participate in society, restored dignity and self-respect, and a sense of belonging within the community. In addition, some members have started to pay more attention to their local environmental hygiene by building pit latrines and waste disposal sites. The ALERT staff involved in this initiative had to change their role from that of a leprosy service provider to a self-care group facilitator, but not all were successful in making this transition. The remaining challenge for the program is sustainability and further development through the National Tuberculosis and Leprosy Control Programme, The Ethiopian National Association for Ex-Leprosy Patients and possibly other organizations too.—Authors' Abstract

Brandsma, J. W. Splinting in leprosy. Indian J. Lepr. **73** (2001) 37–45.

A review is presented to propose guidelines in the use of splinting for common conditions (neuritis, paralysis, and other nerve damage) in persons with leprosy. Indications for splinting, and its duration are explained. Splints for acute neuritis of the ulnar, median, common peroneal, and posterior tibial nerves are discussed. Splints used for motor nerve paralysis such as mobile claw hand, stiff claw hand, lag in assisted extension, flexor tightness, median nerve paralysis, radial nerve paralysis, and common peroneal nerve paralysis are also discussed.—Trop. Dis. Bull.

Courtright, P., Sung-Hwa Kim, Narong Tungpakorn, Byeong-Hee Cho, Young-Kyu Lim, Hyun-Ji Lee and Lewallen, S. Lagophthalmos surgery in leprosy: findings from a population-based survey in Korea. Lepr. Rev. **72** (2001) 285–291.

Lagophthalmos continues to be a serious problem in cured leprosy patients. We conducted a population-based survey of lagophthalmos surgical coverage (LSC), barriers to lagophthalmos surgery and outcome of lagophthalmos surgery in leprosy patients in South Korea. In our survey, there were 60 patients with lagophthalmos who had needed surgery (>5 mm gap), 34 of whom had received surgery, resulting in a lagophthalmos surgery coverage of 57%. Among the 34 patients who had received lagophthalmos surgery, 18 needed further surgery. Among those who had never had surgery, none of the demographic indicators predicted surgical uptake; the primary reason given for failure to have surgery was lack of knowledge about it. Outcome of surgery (by eye) showed that 29% of eyes still had a gap of 5 mm or more. The frequency of symptoms (tearing, blurring of vision, pain, etc.) was high. Even in settings with a good eye care infrastructure, such as Korea, uptake of surgery can still be low and results may not be satisfactory to patients. There is need for practical guidelines for leprosy control programs in the areas of a) patient recognition, b) patient education, c) monitoring the uptake of surgery, and d) monitoring the outcome of surgery to ensure the best possible outcome.—Authors' Abstract

Cross, H. and Newcombe, L. An intensive self care training programme reduces admissions for the treatment of plantar ulcers. Lepr. Rev. **72** (2001) 276–284.

This paper describes, in detail, an intensive 14-day self care training program that is conducted at Lalgadh Leprosy Services Centre in Nepal. An evaluation of the program was undertaken in which hospital admission for infected plantar ulceration was the outcome measure. It was found that those who had undertaken the program were less likely to have been admitted for hospital treatment in a 3-month follow-up period ($\chi^2 = 5.1$, $p = 0.02$). An odds ratio of 1:1.8 (95% CI = 0.15–0.01) was also calculated. This paper presents an overview of the issues related to impairment, a description of the self care training program, an analysis of the evaluation results and a discussion of the findings.—Authors' Abstract

Grimaud, J., Verchot, B., Chapuis, R., Blum, L. and Millan, J. Diagnosis of neuropathy in leprosy in Senegal. Med. Mal. Infect. **31** (2001) 89–91.

To detect early nerve damage in leprosy (caused by *Mycobacterium leprae*), median nerve function was determined in 108 leprosy patients in Senegal by tests on the skin of the hand. Electromyography showed that a test with a nylon thread (0.2 g) was more sensitive than a test with a 0.5 g thread or a prick test. An ether test was not considered suitable under the climatic conditions of Senegal.—Trop. Dis. Bull.

Van Den Broek, J., Van Jaarsveld, T., De Rijk, A., Samson, K. and Patrobas, P. Capture-recapture method to assess the prevalence of disabled leprosy patients. Lepr. Rev. **72** (2001) 292–301.

The capture-recapture technique was applied in estimating the prevalence of disabled leprosy patients in four states in northern Nigeria. A two-sample capture-recapture method using data from hospital admissions during 1997 and 1998 in three leprosy referral hospitals and from a sample survey on leprosy patients with disabilities in the clinics in 1999 was used. In the sample, 1395 former leprosy patients were found and 393 had a disability. Of these 393 patients, 47 had been admitted during 1997 and 1998 to one of three leprosy referral hospitals. In these hospitals, 151 individuals from the 24 study Local Government Areas (LGA) in four states of northern Nigeria were admitted in 1997 and 1998. Using the Peterson estimator, we calculated the number of unknown disabled leprosy patients in the studied LGAs to be 1262 (95% confidence interval 991–1533). This was nearly four times greater than the field reported figure. The capture-recapture method can be applied in a leprosy care program. Limitations of the method are the completeness of reporting after invitation in the field, as well as the probable biased sample of leprosy patients admitted to the hospital. Our finding implies that relying on patients to report for prevention of disabilities and rehabilitation to the clinics, causes the real size of the problem to be underestimated by a factor of 3–4. We recommend the use of a special 'care' register for disabled leprosy patients to better address their needs for prevention of disabilities and rehabilitation.—Authors' Abstract

Other Mycobacterial Diseases and Related Entities

Arnadottir, T. Tuberculosis: trends and the twenty-first century. Scand. J. Infect. Dis. **33**(2001) 563–567.

The global burden of tuberculosis is enormous, even if estimates are somewhat uncertain. The forces counteracting control measures, namely demographic factors, drug resistance, HIV, migration, poverty and marginalization, are enormous as well. With accelerated reforms in tuberculosis programs, important progress can be made toward the control of tuberculosis early in the 21st century. This is confirmed by studying reports from countries where control measures have been implemented

and sustained. Well-functioning programs can make good use of technological progress, such as improved tools for diagnosis and treatment, when these become available at an affordable cost. It is important now to use the opportunity of increased resources in order to reform tuberculosis programs. The biggest impact on global tuberculosis control in the 21st century can be made in Asia. Success in this part of the world depends on political commitment. Elsewhere, the main forces counteracting control measures are HIV in Africa and multidrug resistance in parts of Europe and the former Soviet Union. Here solutions are still on the drawing board. The long time frame for tuberculosis control when using the currently recommended strategy, the uncertain impact of "improved" tools on this time frame and the constant threat that political commitment will not be sustained are reasons why field-workers look toward new technology in the hope of progress in vaccine research. Here, the prospects are uncertain and the forecasted time frame is long. Skeptics even doubt that an effective vaccine can be developed. However, when predicting progress, it is important to realize that it is for the most part unpredictable.—Author'sAbstract

Baatz, J., Tonessen, B., Prada, J. and Pleyer, U. Thalidomide inhibits leukocyte-endothelium interaction in endotoxin-induced uveitis. Opthalmic Res. **33** (2001) 256–263.

To investigate the effects of thalidomide on leukocyte-endothelium interaction in iris vessels of rats with an endotoxin-induced uveitis (EIU), intravital fluorescence microscopy was used to quantify leukocyte adhesion to the vascular endothelium of iris venules in Lewis rats at 2, 4, 8 and 24 h after induction of EIU. Animals (n = 84) received a single intraperitoneal dose of either thalidomide (80 mg/kg body weight) or prednisolone (10 mg/kg body weight). Both drugs significantly reduced firm adhesion of leukocytes at 4, 8 and 24 h. Thalidomide caused earlier suppression of leukocyte rolling than prednisolone (4 vs. 8 h). TNF-alpha plasma levels peaked at 2 h and were not significantly reduced in any group compared with controls. Cell count and protein concentration in aqueous humor were significantly reduced by prednisolone and thalidomide at 24 h ($p < 0.05$). Thalidomide exerts its anti-inflammatory effects by an inhibition of leukocyte-endothelium interaction. Compared with prednisolone, thalidomide shows earlier inhibition of leukocyte rolling, indicating modulation of adhesion molecule expression and/or function.—Authors' Abstract

Barr, R. G., Diez-Roux, A. V., Knirsch, C. A. and Pablos-Mendez, A. Neighborhood poverty and the resurgence of tuberculosis in New York City, 1984–1992. Am. J. Public Health **91** (2001) 1487–1493.

Objectives: The resurgence of tuberculosis (TB) in New York City has been attributed to AIDS and immigration; however, the role of poverty in the epidemic is unclear. We assessed the relation between neighborhood poverty and TB at the height of the epidemic and longitudinally from 1984 through 1992. Methods: Census block groups were used as proxies for neighborhoods. For each neighborhood, we calculated TB and AIDS incidence in 1984 and 1992 with data from the Bureaus of Tuberculosis Control and AIDS Surveillance and obtained poverty rates from the census. Results: For 1992, 3,343 TB cases were mapped to 5,482 neighborhoods, yielding a mean incidence of 46.5 per 100,000. Neighborhood poverty was associated with TB (relative risk = 1.33; 95% confidence interval = 1.30, 1.36 per 10% increase in poverty). This association persisted after adjustment for AIDS, proportion foreign-born, and race/ethnicity. Neighborhoods with declining income from 1980 to 1990 had larger increases in TB incidence than did neighborhoods with increasing income. Conclusions: Leading up to and at the height of the TB epidemic in New York City, neighborhood poverty was strongly associated with TB incidence. Public health interventions should target impoverished areas.—Authors' Abstract

Basilio-de-Oliveira, C., Eyer-Silva, W. A., Valle, H. A., Rodrigues, A. L., Pinheiro Pimentel, A. L. and Morais-De-Sa, C. A. Mycobacterial spindle cell pseudotumor of the appendix vermiformis in a patients with aids. Braz. J. Infect. Dis. **5** (2001) 98–100.

Mycobacterial pseudotumor (MP) is a rare pathologic presentation of both *Mycobacterium tuberculosis* and non-tuberculous mycobacterial disease, hitherto reported to occur only in immunosuppressed patients with or without human immunodeficiency virus infection. This lesion shares close pathologic resemblance to certain mesenchymal neoplasms, particularly Kaposi's sarcoma (KS), from which it must be properly differentiated due to distinct prognosis and therapy. We report a case of MP obliterating the lumen of the appendix vermiformis in a 34-year-old patient who died of complications of AIDS at our hospital in Rio de Janeiro. A total of 24 cases of MP (including our patient) have been described in the literature. MP has been found especially in lymph nodes, but extranodal lesions have been described in the skin, spleen, lung, bone marrow, brain and, in our patient, the appendix vermiformis. We offer a review of the other 23 published case reports of MP in both HIV-infected and uninfected patients and discuss the pathologic features that differentiate MP from KS.—Authors' Abstract

Bentoucha, A., Robert, J., Dega, H., Lounis, N., Jarlier, V. and Grosset, J. Activities of new macrolides and fluoroquinolones against *Mycobacterium ulcerans* infection in mice. Antimicrob. Agents Chemother. **45** (2001) 3109–3112.

Mice infected in the left hind footpad with 5 log(10) acid-fast bacilli of *Mycobacterium ulcerans* were divided into an untreated control group and 17 treatment groups that received one of the following regimens for 4 weeks (all doses in milligrams per kilogram): 100 mg of azithromycin (AZM), 100 mg of clarithromycin (CLR), or 50 mg of AZM for a duration of 5 days a week (daily), three times a week, or once weekly. In addition, the following regimens were administered daily: 100 mg of telithromycin (TLM), sparfloxacin (SPX), or moxifloxacin (MOX); 200 mg of levofloxacin (LVX); 100 mg of streptomycin (STR) or amikacin (AMK); 10 mg of rifampin (RIF); and the combination of 10 mg of RIF and 100 mg of AMK (RIF+AMK). After completion of treatment, mice were observed for 30 weeks. The effectiveness of treatment regimens was assessed in terms of the delay in median time to footpad swelling in treated mice compared with that in the untreated controls. Clear-cut bactericidal activity, i.e., an observed delay in footpad swelling that exceeded the period of treatment, was observed in the STR-, AMK-, and RIF+AMK-treated mice. However, all mice treated with either AMK or STR alone had swollen footpads before the end of the 30-week observation period, suggesting regrowth of *M. ulcerans*. In contrast, 50% of the mice treated with the RIF+AMK combination exhibited no lesion even after 30 weeks, suggesting a cure. The remaining regimens could be assigned to one of three groups: (i) no activity (50 mg of AZM, 100 mg of AZM thrice weekly, TLM, and LVX); (ii) bacteriostatic activity, i.e., a delay in footpad swelling shorter than the 4-week treatment duration (100 mg of AZM daily or once weekly, CLR thrice or once weekly, and MOX); or (iii) weak bactericidal activity (CLR daily and SPX). The RIF+AMK combination and possibly RIF+STR warrant further study for the treatment of *M. ulcerans* infection in humans.—Authors' Abstract

Borkow, G., Weisman, Z., Leng, Q., Stein, M., Kalinkovich, A., Wolday, D. and Bentwich, Z. Helminths, human immunodeficiency virus and tuberculosis. Scand. J. Infect. Dis. **33** (2001) 568–571.

Helminth infections affect over a quarter of the world's population, especially in the developing countries. These long-lasting parasitic infections cause widespread immune activation and dysregulation, a dominant Th2 cytokine immune profile and an immune hyporesponsiveness state. Considering these profound immune changes and the similar geographic distributions of

helminthic infections, HIV and tuberculosis (TB), we suggest that helminthic infections play a major role in the pathogenesis of AIDS and TB. They apparently make the host more susceptible to infection by HIV and *Mycobacterium tuberculosis*, and impair his/her ability to generate protective immunity against both infections. The implication of these ideas is that without eradication of helminth infections and/or modulation of the immune changes that they cause, HIV and TB vaccines may fail to confer protection against their respective infections in helminth-endemic areas.—Authors' Abstract

Brown-Elliott, B. A., Wallace, Jr., R. J., Blinkhorn, R., Crist, C. J. and Mann, L. B. Successful treatment of disseminated *Mycobacterium chelonae* infection with linezolid. Clin. Infect. Dis. **33** (2001) 1433–1434.

We describe a 57-year-old man with steroid-dependent myasthenia gravis and progressive ulcerating leg nodules due to clarithromycin-resistant *Mycobacterium chelonae*. The patient was successfully treated with linezolid.—Authors' Abstract

Casanova, J. L. Mendelian susceptibility to mycobacterial infection in man. Swiss Med. Wkly. **131** (2001) 445–454.

Selective susceptibility to weak pathogenic mycobacteria, such as bacillus Calmette-Guerin (BCG) vaccine and environmental non-tuberculous mycobacteria (NTM), has long been suspected to be a Mendelian disorder, but its molecular basis remained elusive. Recently, mutations in the interferon-gamma receptor ligand-binding chain (IFN-gamma R1), interferon-gamma receptor signaling chain (IFN-gamma R2), Signal Transducer and Activator of Transcription-1 (STAT-1), interleukn-12 p40 subunit (IL-12 p40), and interleukin-12 receptor beta 1 chain (IL-12R beta 1) genes have been identified in a number of patients with severe BCG or NTM infection. Dominant or recessive alleles causing complete or partial cellular defects have been found to define nine different inheritable disorders. Although genetically distinct, these conditions are immunologically related and highlight the essential role of interferon gamma-mediated immunity in the control of mycobacteria in man. The genetic and immunologic heterogeneity of this syndrome makes accurate diagnosis challenging but vital, as decisions about the most appropriate treatment are best taken based on an accurate molecular diagnosis.—Author's Abstract

Chemlal, K., Huys, G., Fonteyne, P. A., Vincent, V., Lopez, A. G., Rigouts, L., Swings, J., Meyers, W. M. and Portaels, F. Evaluation of PCR-restriction profle analysis and IS2404 restriction fragment length polymorphism and amplified fragment length polymorphism fingerprinting for identification and typing of *Mycobacterium ulcerans* and *M. marinum*. J. Clin. Microbiol. **39** (2001) 3272–3278.

Mycobacterium ulcerans and *M. marinum* are emerging necrotizing mycobacterial pathogens hat reside in common reservoirs of infection and exhibit striking pathophysiological similarities. Furthermore, the interspecific taxonomic relationship between the two species is not clear as a result of the very high phylogenetic relatedness (i.e., >99.8% 16S rRNA sequence similarity), in contrast to only 25% to 47% DNA relatedness. To help understand the genotypic affiliation between these two closely-related species, we performed a comparative analysis including PCR restriction profile analysis (PRPA), IS2404 restriction fragment length polymorphism (RFLP), and amplified fragment length polymorphism (AFLP) on a set of *M. ulcerans* (n = 29) and *M. marinum* (n = 28) strains recovered from different geographic origins. PRPA was based on a triple restriction of the 3′ end region of 16S rRNA, which differentiated *M. ulcerans* into three types; however, the technique could not distinguish *M. marinum* from *M. ulcerans* isolates originating from South America and southeast Asia. RFLP based on IS2404 produced six *M. ulcerans* types related to six geographic regions and did not produce any band with *M. marinum*, confirming the previous findings of Chemlal, et al. (K. Chem-

lal, K. DeRidder, P. A. Fonteyne, W. M. Meyers, J. Swings, and F. Portaels, Am. J. Trop. Med. Hyg. (2001) *64* 270–273. AFLP analysis resulted in profiles which grouped *M. ulcerans* and *M. marinum* into two separate clusters. The numerical analysis also revealed subgroups among the *M. marinum* and *M. ulcerans* isolates. In conclusion, PRPA appears to provide a rapid method for differerntiating the African *M. ulcerans*-type from other geographical types, but is unsuitable for interspecific differentiation of *M. marinum* and *M. ulcerans.* In comparison, whole-genome techniques such as IS 2404-RFLP and AFLP appear to be far more useful in discriminating between *M. marinum* and *M. ulcerans,* and may, thus, be promising molecular tools for the differential diagnosis of infections caused by these two species.—Authors' Abstract

Çiçek Saydam, C., Çavusoğlu, C., Burhanoğlu, D., Özkalay, N. and Bilgiç, A. *In vitro* activity of amoxicillin-clavulanic acid and clarithromycin on *Mycobacterium tuberculosis* by using E-test. Turk. J. Infec. **14** (2000) 485–489.

This study aims to determine the *in vitro* susceptibility of *M. tuberculosis* to amoxicillin-clavulanic acid and clarithromycin [Turkey]. Activities of the 2 drugs on 71 clinical isolates of *M. tuberculosis* were determined by using E-test. Fifty-four out of 71 clinical isolates were susceptible (minimum inhibitory concentration at which 90% of the organism are inhibited (MIC_{90}) ≤2 μg/ml) to clarithromycin, while 5 isolates were intermediately susceptible (MIC_{90} = 8 μg/ml). 12 isolates were resistant (MIC_{90} ≥16 μg/ml) to clarithromycin. Thirty-five of 71 *M. tuberculosis* isolates were susceptible to amoxicillin-clavulanic acid, while 9 isolates exhibited intermediate susceptibility. Twenty-seven isolates were resistant to amoxicillin-clavulanic acid.—Trop. Dis. Bull.

Crick, D. C., Mahapatra, S. and Brennan, P. J. Biosynthesis of the arabinogalactan-peptidoglycan complex of *Mycobacterium tuberculosis.* Glycobiology **11** (2001) 107R–118R.

The compositional complexity of the mycobacterial cell envelope differentiates Mycobacterium species from most other prokaryotes. Historically, research in this area has focused on the elucidation of the structure of the mycobacterial cell envelope with the result that the structures of the mycolic acid-arabinogalactan-peptidoglycan complex from *M. tuberculosis* are fairly well understood. However, the current impetus for studying *M. tuberculosis* and other pathogenic mycobacteria is the need to identify targets for the development of new drugs. Therefore, emphasis has been shifting to the study of cell envelope biosynthesis and the identification of enzymes that are essential to the viability of *M. tuberculosis.* The publication of the complete *M. tuberculosis* genome in 1998 has greatly aided these studies. To date, 13 enzymes involved in the synthesis of the arabinogalactan-peptidoglycan complex of *M. tuberculosis* have been identified and at least partially characterized. Eleven of these enzymes were reported subsequent to the publication of the *M. tuberculosis* genome, a clear indication of the rapid evolution of knowledge stimulated by the sequencing of the genome. In this article we review the current understanding of *M. tuberculosis* arabinogalactan-peptidoglycan structure and biosynthesis.—Authors' Abstract

Dieli, F., Troye-Blomberg, M., Ivanyi, J., Fournie, J. J., Krensky, A. M., Bonneville, M., Peyrat, M. A., Caccamo, N., Sireci, G. and Salerno, A. Granulysin-dependent killing of intracellular and extracellular *Mycobacterium tuberculosis* by Vgamma9/Vdelta2 T lymphocytes. J. Infect. Dis. **184** (2001) 1082–1085.

Contribution of Vgamma9/Vdelta2 T lymphocytes to immune protection against *Mycobacerium tuberculosis* is still a matter of debate. It was reported earlier that Vgamma9/Vdelta2 T lymphocytes kill macrophages harboring live *M. tuberculosis* through a granule-dependent mechanism that results in killing of intracellular bacilli. This study found that Vgamma9/Vdelta2 T lymphocytes reduce the viability of both extracellular and intracellular *M. tuberculo-*

sis. Granulysin and perforin, both detected in Vgamma9/Vdelta2 T ymphocytes, play a major role, which indicates that Vgamma9/Vdelta2 T lymphocytes directly contribute to a protective host response against *M. tuberculosis* infection.—Authors' Abstract

Dobos, K. M., Small, P. L., Deslauriers, M., Quinn, F. D. and King, C. H. *Mycobacterium ulcerans* cytotoxicity in an adipose cell model. Infect. Immun. **69** (2001) 7182–7186.

An adipose cell (SW872) model was developed to observe cellular necrosis and apoptosis upon *Mycobacterium ulcerans* infection and treatment with mycobacterial exudate. Apoptosis was likely due to secreted proteins, while necrosis was likely due to mycolactone. Our data suggest that additional factors in *M. ulcerans* may be involved in Buruli ulcer pathogenesis.—Authors' Abstract

Global Alliance for TB Drug Development. Tuberculosis. Scientific blueprint for tuberculosis drug development. Tuberculosis **81** (2001) 1–52.

The Global Alliance for TB Drug Development is dedicated to closing the R&D gaps. However, advances cannot be made without investment by national and international health organizations, private sector pharmaceutical and biotechnology firms, foundations, and others. Their support is needed to develop a broad portfolio of promising candidates with a special emphasis on developing fast-track and/or sterilizing drugs. Funding agencies and research organizations must devote significant resources in the short term to close the gaps in the R&D value chain and to leverage the strengths available. Fortunately, the need, the expertise, and the enthusiasm exist. By combining resources into R&D efforts to discover and develop a broad portfolio of promising candidates, the Global Alliance and its sponsors can make a vitally important contribution to improved control and the eventual elimination of tuberculosis from every country of the world.—Authors' Abstract

Grover, J. K., Vats, V., Gapalakrishna, R. and Ramam, M. Thalidomide: a relook. Natl. Med. J. India. **13** (2000) 132–141.

Thalidomide was synthesized in 1954 in the former West Germany and marketed as a sedative in over 46 countries until the early 1960s. Owing to serious teratogenic effects, the drug was withdrawn from the market in 1961. A chance observation suggested the utility of thalidomide in erythema nodosum leprosum (ENL). After many controlled and uncontrolled trials were published, the World Health Organization recommended its use in ENL. The Food and Drug Administration, USA, approved it for use in ENL in July 1998. Only established and well-defined studies conducted to substantiate the efficacy of thalidomide have been included in this review. Thalidomide is considered the drug of choice for the treatment of ENL, but for other conditions, it is recommended only when resistance to the currently available form of therapy is encountered. Once the anti-inflammatory, immuno-modulatory, anti-TNF-alpha and anti-angiogenic properties of thalidomide were discovered, it was also tried in AIDS and related wasting, apthous ulcers, microsporidiosis and Kaposi's sarcoma. Thalidomide has no clinical place as an immunosuppressant in solid organ transplantation. However, it has a therapeutic role in graft-verus-host disease. Among the dermatological conditions, thalidomide has been found to be effective in systemic lupus erythematosus, discoid lupus erythematosus, actinic prurigo and prurigo nodularis. Used correctly, it is a safe and effective medicine (except for its teratogenic potential and delayed neuropathy) in a variety of disease conditions.—Authors' Abstract

Hadjichristodoulou, C., Christie, P. and O'Brien, S. Pulmonary tuberculosis and deprivation in hospitalised patients in Scotland. Eur. J. Epidemiol. **17** (2001) 85–87.

During the last decades tuberculosis reemerged in almost all the world, in both developed and developing countries. Many risk factors were implicated to explain the

re-emergences, including the HIV epidemic. The aim of the study was to explore if tuberculosis is related with poverty in Scotland utilizing routinely collected hospital discharge data for patients with pulmonary tuberculosis and postcode-derived Carstairs deprivation scores. The Carstairs and Morris index is composed of four indicators which were judged to represent material disadvantage in the population. A positive correlation was found between the cumulative incidence rate for hospitalized patients within each Health Board and the Carstairs deprivation score ($r = 0.16$, $p < 0.01$). A similar correlation was found between the cumulative incidence rate and the deprivation scores within each postcode sector ($r = 0.47$, $p < 0.0001$). These results support findings by other researchers that poverty and tuberculosis are related, and might be one explanation for the recent re-emergence of tuberculosis.—Authors' Abstract

Hanekom, W. A., Hughes, J., Haslett, P. A., Apolles, P., Ganiso, V., Allin, R., Goddard, E., Hussey, G. D. and Kaplan, G. The immmunomodulatory effects of thalidomide on human immunodeficiency virus-infected children. J. Infect. Dis. **184** (2001) 1192–1196.

The safety and immune effects of low-dose thalidomide treatment (3 mg/kg/day for 28 days) were evaluated in a study involving 8 South African human immunodeficiency virus (HIV)-infected children. The children were 7–69 months old and in disease stages A1–C3. Thalidomide therapy did not affect virus load, even though none of the children was receiving antiretroviral therapy. Thalidomide stimulated CD8+ T cells in peripheral blood, which increased expression of the activation markers CD38 and human leukocyte antigen DR and of the memory cell marker CD45R0. The frequency of HIV gag-specific CD8+ T cells in peripheral blood increased in 3 of 4 children who were evaluated during treatment with thalidomide. Clinical adverse events were mild. In this study, thalidomide was found to be safe and well-tolerated and caused significant immunomodulation at a low dose. This is the first report describing use of an oral drug that may enhance HIV-specific CD8+ T cell function in HIV-infected children.—Authors' Abstract

Kenet, G., Wardi, J., Avni, Y., Aeed, H., Shirin, H., Zaidel, L., Hershkoviz, R. and Bruck, R. Amelioration of experimental colitis by thalidomide. Isr. Med. Assoc. J. **3** (2001) 644–648.

Rectal administration of iodoacetamide induces colitis by blocking sulphhydryl groups and generating inflammatory mediators. Thalidomide, a non-barbiturate hypnotic, also has an anti-inflammatory effect, presumably by suppressing the production of tumor necrosis factor alpha. In patients with Crohn's disease, neutralization or suppression of TNF alpha reduces inflammation. Objectives: To evaluate the effects of thalidomide in a model of experimental colitis. Methods: Colitis was induced in rats by intracolonic administration of 3% iodoacetamide. In the treatment group, thalidomide 50 mg/kg was given daily by gavage and continued for 7 days until the rats were sacrificed. Their colons were then processed for wet weight, lesion area, weight of mucosal scraping, myeloperoxidase activity and histology. Serum levels of TNF were determined. Results: Colonic wet weight, lesion area, myeloperoxidase activity and serum levels of TNF alpha were significantly lower ($P < 0.05$) in the treatment group (iodoacetamide + thalidomide) than the control group (iodoacetamide only). Histologically, colonic inflammation in the treated group was markedly decreased. Conclusions: Thalidomide effectively decreases colitis induced by iodoacetamide. The mechanism is probably associated with inhibition of TNF alpha, and should be further studied.—Authors' Abstract

Lounis, N., Truffot-Pernot, C., Bentoucha, A., Robert, J., Ji, B. and Grosset, J. Efficacies of clarithromycin regimens against *Mycobacterium xenopi* in mice. Antimicrob. Agents Chemother. **45** (2001) 3229–3230.

Mice were infected intravenously with 3.5×10^7 CFU of *Mycobacterium xenopi*

and treated with various clarithromycin-containing regimens or left untreated for 4 weeks. All nine of the clarithromycin-containing regimens reduced the CFU counts to the levels below the pretreatment values, indicating that these regimens had a bactericidal effect on *M. xenopi* in mice. The rifampin-isoniazid-ethambutol regimen was significantly less bactericidal than clarithromycin alone or clarithromycin-containing combined regimens.—Authors' Abstract

Meierhofer, C., Dunzendorfer, S. and Widermann, C. J. Theoretical basis for the activity of thalidomide. BioDrugs **15** (2001) 681–703.

The revival of thalidomide began shortly after the drug was withdrawn from the market because of its teratogenic properties. Therapeutic effects of thalidomide were found accidentally in leprosy patients with erythema nodosum leprosum (ENL). Subsequent research widened the understanding of the activity of thalidomide and, with improved methodology and the augmented background knowledge of immunology, it was possible to interpret the properties of thalidomide more coherently. Effects on tumor necrosis factor-alpha (TNF-alpha) release play an important role in the ability of thalidomide to affect the immune system. Alteration of synthesis and release of cytokines such as interleukin (IL)-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 and interferon-gamma is involved in the complex mechanisms of thalidomide. Thalidomide targets leucocytes, endothelial cells and keratinocytes, affecting them in a different manner and at different cellular levels. Changes in the density of adhesion molecules alter leucocyte extravasation and the inflammatory response in the tissue involved. Several mechanisms for the teratogenic action of thalidomide are currently under review, but this mode of action of the drug still remains unclear and we review evidence-based hypotheses for the teratogenicity of thalidomide. Thalidomide shows significant clinical impact in several diseases, such as ENL in lepromatous leprosy, chronic graft-versus-host disease, systemic lupus erythematosus, sarcoidosis, aphthous lesions in HIV infection, wasting syndrome in chronic illness, inflammatory bowel disease, multiple myeloma and some solid tumors. In 1998 the U.S. Food and Drug Administration approved thalidomide exclusively for the treatment of ENL, and strict conditions were stipulated for its use in order to prevent teratogenic adverse effects. However, despite the promising findings of thalidomide at the molecular level, namely its anti-TNF-alpha properties and its intercalation with DNA, and activity in clinical trials, there is still a great need for more intensive research.—Authors' Abstract

Morsczeck, C., Berger, S. and Plum, G. The macrophage-induced gene (mig) of *Mycobacterium avium* encodes a medium-chain acyl-coenzyme A synthetase. Biochem. Biophys. Acta **31** (2001) 59–65.

The macrophage-induced gene (mig) of *Mycobacterium avium* has been associated with virulence, but the functions of the gene product were still unknown. Here we have characterized the mig protein by biochemical methods. A plasmid with a histidine-tagged fusion protein was constructed for expression in *Escherichia coli.* Mig was detected as a 60-kDa protein after expression and purification of the recombinant gene product. The sequence of the fusion gene and of the parent gene in *M. avium* were re-examined. This confirmed that the mig gene encodes a 550 amino acid protein (58 kDa) instead of a 295 amino acid protein (30 kDa) as predicted before. The 550 amino acid mig exhibits a high degree of homology to bacterial acyl-CoA synthetases. Two artificial 30-kDa derivatives of mig were expressed and purified as histidine-tagged fusion proteins in *E. coli.* These proteins and the 58.6-kDa histidine-tagged mig protein were analyzed for activity with an acyl-CoA synthetase assay. Among the three investigated proteins, only the 58.6-kDa mig exhibited detectable activity as an acyl-CoA synthetase (EC 6.2.1.3) with saturated medium-chain fatty acids, unsaturated long-chain fatty acid and some aromatic carbon acids as substrates. Enzymatic activity could be inhibited by 2-hydroxydodecanoic acid, a typical inhibitor of medium-

chain acyl-CoA synthetases. We postulate a novel medium-chain acyl-CoA synthetase motif. We have investigated the biochemical properties of mig and suggest that this enzyme is involved in the metabolism of fatty acid during mycobacterial survival in macrophages.—Authors' Abstract

Nishimoto, A., Narita, K., Ohmoto, S., Takahashi, Y., Yoshizumi, S., Yoshida, T., Kado, N., Okezaki, E. and Kato, H. Studies on macrolide antibiotics I. Synthesis and antibacterial activity of erythromycin A 9-O-substituted oxime ether derivatives against *Mycobacterium avium* complex. Chem. Pharm. Bull. (Tokyo) **49** (2001) 1120–1127.

A series of erythromycin A 9-O-substituted oxime ether derivatives have been synthesized and evaluated for antibacterial activity against *Mycobacterium avium* complex (MAC) and *Staphylococcus aureus.* These compounds possessed stronger *in vitro* activity against MAC including macrolide-resistant strains than clarithromycin, although *in vitro* antibacterial activities of these compounds were less than that of 2 against *Staphylococcus aureus.* Our studies found that several factors contribute to the antibacterial activity against MAC. The length and spatial orientation of the substituent at 9-position were found to significantly influence the anti-MAC activity, especially against macrolide-resistant strains. Of all the compounds prepared, erythromycin A 9-[O-(4-phenylbutyl)oximel] (12q) and erythromycin A 9-[O-(3-phenoxypropyl)oxime] (12t) possessed 16 times stronger antibacterial activity than 2 against clarithromycin-resistant strains. Surprisingly, the minimum inhibitory concentrations (MICs) of 12q and 12t against the resistant strains were almost the same as those against the susceptible strains. These results suggest that the erythromycin A 9-O-substituted oxime ether derivatives would be promising macrolide antibiotics.—Authors' Abstract

Onat, D., Stahl, W. and Sies, H. Stimulation of gap junctional intercellular communication by thalidomide and thalidomide analogs in human fetal skin fibroblasts (HFFF2) and in rat liver epithelial cells (WB-F344)(1). Biochem. Pharmacol. **62** (2001) 1081–1086.

Gap junction channels maintain cell-cell communication and are essential for the coordination of tissues, playing a pivotal role in embryonal development. Gap junctional intercellular communication (GJIC), studied here in human fetal skin fibroblasts (HFFF2) and in rat liver epithelial cells (WB-F344), was almost doubled upon exposure to thalidomide (10 μM) in the presence of NADH or NADPH (20 μM). Neither in HFFF2 nor in WB-F344 cells did any detectable alteration in GJIC occur with the thalidomide analog EM 16 (10 μM), known as a non-teratogenic compound. The thalidomide analog EM 364 (10 μM) increased GJIC without prior metabolic activation. It is suggested that GJIC modification may be related to the pharmacological and toxicological properties of thalidomide.—Authors' Abstract

Passmore, J. S., Glashoff, R. H., Lukey, P. T. and Ress, S. R. Granule-dependent cytolysis of *Mycobacterium tuberculosis*-infected macrophages by human gammadelta+ T cells has no effect on intracellular mycobacterial viability. Clin. Exp. Immunol. **126** (2001) 76–83.

One of the most important effector functions of activated gammadelta+ T cells in tuberculosis is their strong cytolytic activity against a variety of target cells, including *M. tuberculosis*-infected macrophages. In the present study, we investigated the relationship between the mechanism of cytolysis utilized by gammadelta+ CTL and intracellular *M. tuberculosis* survival using a panel of cytolytic human *M. tuberculosis*-specific is gammadelta+ CTL clones. Cytolysis mediated by the gammadelta+ T-cell clones was found to be Ca2+-dependent, sensitive to Cyclosporin A, and was completely abrogated following Sr2+-induced de-granulation of the gammadelta+ T cell effectors. These data demonstrate that gammadelta+ T-cell-mediated cytoxicity was mediated via the granule exocytosis/perforin pathway. Despite significant cytolytic

activity against mycobacteria infected U937 cells, the gammadelta+ CTL clones had no impact on the survival of intracellular *M. tuberculosis.*—Authors' Abstract

Ragno, S., Romano, M., Howell, S., Pappin, D. J., Jenner, P. J. and Colston, M. J. Changes in gene expression in macrophages infected with *Mycobacterium tuberculosis*: a combined transcriptomic and proteomic approach. Immunology **104** (2001) 99–108.

We investigated the changes which occur in gene expression in the human macrophage cell line, THP1, at 1, 6 and 12 hr following infection with *Mycobacterium tuberculosis.* The analysis was carried out at the transcriptome level, using microarrays consisting of 375 human genes generally thought to be involved in immunoregulation, and at the proteomic level, using two-dimensional gel electrophoresis and mass spectrometry. The analysis of the transcriptome using microarrays revealed that many genes were up-regulated at 6 and 12 hr. Most of these genes encoded proteins involved in cell migration and homing, including the chemokines interleukin (IL)-8, osteopontin, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1alpha (MIP-1alpha), regulated on activation, normal, T-cell expressed and secreted (RANTES), MIP-1beta, MIP-3alpha, myeloid progenitor inhibitory factor-1 (MPIF-1), pulmonary and activation regulated chemokine (PARC), growth regulated gene-beta (GRO-beta), GRO-gamma, MCP-2, I-309, and the T helper 2 (Th2) and eosinophil-attracting chemokine, eotaxin. Other genes involved in cell migration which were up-regulated included the matrix metalloproteinase MMP-9, vascular endothelial growth factor (VEGF) and its receptor F1k-1, the chemokine receptor CCR3, and the cell adhesion molecules vesicular cell adhesion molecule-1 (VCAM-1) and integrin a3. In addition to the chemokine response, genes encoding the proinflammatory cytokines IL-lbeta (showing a 433-fold induction), IL-2 and tumor necrosis factor-alpha (TNF-alpha), were also found to be induced at 6 and/or 12 hr. It was more difficult to detect changes using the proteomic approach. Nevertheless, IL-1beta was again shown to be strongly up-regulated. The enzyme manganese superoxide dismutase was also found to be strongly up-regulated; this enzyme was found to be macrophage-derived, rather than *M. tuberculosis.* The heat-shock protein hsp27 was found to be down-regulated following infection. We also identified a mycobacterial protein, the product of the atpD gene (thought to be involved in the regulation of cytoplasmic pH) in the infected macrophage extracts.—Authors' Abstract

Ridder, G. J., Fradis, M. and Lohle, E. Cheilitis granulomatosa Meischer: treatment with clofazimine and review of the literature. Ann. Otol. Rhinol. Laryngol. **110** (2001) 964–967.

Cheilitis granulomatosa Miescher is a rare condition of unknown cause characterized by intermittent lip swelling that gradually persists and causes cosmetic deformity. We report the case of a young woman with cheilitis granulomatosa as a monosymptomatic manifestation of Melkersson-Rosenthal syndrome successfully treated by the antileprosy agent clofazimine, and propose clofazimine as an alternative treatment in cases refractory to corticosteroids. The differential diagnosis and current methods of treatment are summarized, and the literature is reviewed and discussed.—Authors' Abstract

Schaeffer, M. L., Agnihotri, G., Volker, C., Kallender, H., Brennan, P. J. and Lonsdale, J. T. Purification and biochemical characterization of the *Mycobacterium tuberculosis* betaketoacyl-acyl carrier protein synthases, KasA, and KasB. J. Biol. Chem. **276** (2001) 47029–47037.

Mycolic acids are vital components of the *Mycobacterium tuberculosis* cell wall, and enzymes involved in their formation represent attractive targets for the discovery of novel anti-tuberculosis agents. Biosynthesis of the fatty acyl chains of mycolic acids involves two fatty-acid synthetic systems, the multifunctional polypeptide FASI,

which performs *de novo* fatty acid synthesis, and the dissociated FASII system, which consists of monofunctional enzymes and acyl carrier protein (ACP) and elongates FASI products to long-chain mycolic acid precursors. In this study, we present the initial characterization of purified KasA and KasB, two beta-ketoacyl-ACP synthase (KAS) enzymes of the *M. tuberculosis* FASII system. KasA and KasB were expressed in *Escherichia coli* and purified by affinity chromatography. Both enzymes showed activity typical of bacterial KASs, condensing an acyl-ACP with malonyl-ACP. Consistent with the proposed role of FASII in mycolic acid synthesis, analysis of various acyl-ACP substrates indicated KasA and KasB had higher specificity for long-chain acyl-ACPs containing at least 16 carbons. Activity of KasA and KasB increased with use of *M. tuberculosis* AcpM suggesting that structural differences between AcpM and *E. coli* ACP may affect their recognition by the enzymes. Both enzymes were sensitive to KAS inhibitors cerulenin and thiolactomycin. These results represent important steps in characterizing KasA and KasB as targets for antimycobacterial drug discovery.—Authors' Abstract

Wayne, L. G. and Sohaskey, C. D. Nonreplicating persistence of *Mycobacterium tuberculosis*. Annu. Rev. Microbiol. **55** (2001) 139–163.

There is ample clinical evidence, as well as evidence from animal experiments, that *Mycobacterium tuberculosis* can persist in tissues for months to decades without replicating, yet with the ability to resume growth and activate disease. Our knowledge of both macrophage physiology and the nature of tuberculous lesions in man and animals suggests that hypoxia is a major factor in inducing nonreplicating persistence (NRP) of tubercle bacilli. *In vitro* models reinforce this conclusion and provide insights into mechanisms that make NRP possible. There is evidence from *in vitro* models that the strategies employed by the bacilli to permit hypoxic NRP include restriction of biosynthetic activity to conserve energy, induction of alternative energy pathways, and stabilization of essential cell components to lessen the need for repair or replacement.—Authors' Abstract